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CERVICAL MYELOPATHY DUE TO OSSIFICATION OF THE POSTERIOR LONGITUDINAL LIGAMENT

A CLINICAL, RADIOLOGICAL AND EVOKED POTENTIALS STUDY IN SIX CHINESE PATIENTS

by Y. L. YU[1], J. C. Y. LEONG[2], D. FANG[2], E. WOO[1], C. Y. HUANG[1] *and* H. K. LAU[2]

(*From the* [1]*Departments of Medicine and* [2]*Orthopaedic Surgery, University of Hong Kong*)

SUMMARY

Six consecutive Chinese patients with cervical myelopathy due to ossification of the posterior longitudinal ligament (OPLL) who presented in a 4 yr period were studied. There were 5 males and 1 female whose mean age at presentation was 67 yrs. The common clinical features were spastic tetraparesis more marked in the lower limbs, spinothalamic and posterior column sensory deficits of varying degree and sphincter disturbance in advanced cases. OPLL was diagnosed on plain radiographs and cord compression ascertained by conventional and computer-assisted myelography. Somatosensory evoked potentials correlated with posterior column signs and appeared to reflect the clinical course following treatment. While the cord pathology in OPLL is similar to that of cervical spondylotic myelopathy (CSM), the pathogenetic mechanism probably differs in one aspect. Compression by OPLL is the determining factor in most cases whereas the combined presence of a congenitally narrow canal and spondylosis is essential for the development of CSM. Other contributory factors include coexisting spondylosis, a congenitally narrow canal, friction between cord and OPLL during neck movement and acute trauma. Cord damage is probably mediated by ischaemia which is due to disturbances of the microcirculation caused by the mechanical factors.

INTRODUCTION

The posterior longitudinal ligament extends from the basiocciput to the sacrum, spreading over the midline and posterior aspect of the vertebral bodies and intervertebral discs. Ossification of the posterior longitudinal ligament (OPLL) may occur and cause spinal cord compression. The first report was published in 1838 by Key, to be followed only by 2 reports in Caucasians (Knaggs, 1925; Oppenheimer, 1942) in the first half of this century.

In 1960 Tsukimoto reported the autopsy findings in a 47-yr-old Japanese with progressive cervical myelopathy due to OPLL extending between C3 and C4

Correspondence to: Dr Y. L. Yu, Department of Medicine, Queen Mary Hospital, Hong Kong.

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vertebral bodies. The paper generated interest among Japanese clinicians, resulting in further reports: 7 cases by Suzuki *et al.* (1962), 1 by Koizumi (1962) and 2 by Terayama *et al.* (1964). Since the review of 18 cases by Onji *et al.* (1967), the number of reports has increased rapidly due to increased recognition. Ono *et al.* (1977) reported the clinical and radiological features in 166 Japanese patients together with autopsy findings in 2. These reports earned OPLL the label of 'Japanese disease' (Breidahl, 1969) and also prompted a nationwide study commissioned by the Investigation Committee of the Japanese Ministry of Public Health and Welfare (1981).

OPLL is rare among Caucasians and only about 25 cases have been reported to date (Key, 1838; Knaggs, 1925; Oppenheimer, 1942; Minagi and Gronner, 1969; Bakay *et al.*, 1970; Forcier and Horsey, 1970; Palacios *et al.*, 1971; Hyman *et al.*, 1977; Resnick *et al.*, 1978; Rozario *et al.*, 1978; Hanna and Watt, 1979). This condition is also scarcely reported in non-Japanese orientals. Soo and Sachdev (1971) described 1 Indian and 1 Chinese, while Chin and Oon (1979) reported from Singapore 10 Chinese, 4 Indian and 2 Malay patients with OPLL. The clinical and radiological data in these reports were, however, sparse. We now report in detail 6 Chinese patients with cervical myelopathy due to OPLL.

PATIENTS AND METHODS

Six consecutive patients with cervical myelopathy due to OPLL attended the University Departments of Medicine and Orthopaedic Surgery between 1982 and 1986. Detailed neurological examination was performed at presentation and follow-up and the clinical features were classified under particular tract dysfunction as follows. (1) Anterolateral column involvement indicated by upper motor neuron signs, disturbance of bladder control, paraesthesiae and impairment of pain and temperature sensation. The signs of 'myelopathy hand' as described by Ono *et al.* (1987) were sought. These consisted of deficient adduction and/or extension of the ulnar 2 or 3 fingers (the finger escape sign), and inability to grip and release rapidly with the fingers (less than 20 times in 10 s). Anterior horn cell damage was considered likely in the presence of weakness and wasting in the hands and brisk tendon reflexes in the upper limbs. (2) Posterior and posterolateral column lesions, including impairment of joint position and vibration sensation. Relevant investigation were performed to exclude as far as possible intrinsic cervical cord diseases.

The patient's functional disability was graded as mild when daily activity was only slightly affected, moderate when there was dependence on aids or help and severe when the patient was bedridden, chairbound or had useless upper extremities. Clinical improvement after treatment was considered marked if the functional status had moved up one grade or more, and slight if there was a less pronounced, although significant, decrease in the severity of symptoms and signs. Deterioration was similarly assessed.

Plain radiographs of the cervical spine in standard projections were obtained. The sagittal diameter (APD) of the cervical canal, defined as the distance between the nearest points of the posterior vertebral body and the spinal lamina, was measured and corrected for magnification at midvertebral and intervertebral levels from C2 to C7. The canal was congenitally narrow if at any midvertebral point the value was less than 12 mm. The extent of OPLL and the acquired APD due to OPLL or spondylosis were documented, the APD at the narrowest point being defined as minimal sagittal diameter (mAPD). Spondylotic changes were graded as mild, moderate and marked. Metrizamide myelography was performed to ascertain the site and degree of cord compression. Computed tomography (CT) of the cervical region, either with intrathecal contrast (computer-assisted myelography) or without, was performed using the GE CT 8800 scanner. The images were obtained perpendicular to the axis of the spine and at window settings adjusted according to the method described by Siebert *et al.* (1981).

Somatosensory evoked potentials (SEPs) were performed using the ST 10 stimulator and Medelec MS6 machine. Electrical stimuli (square-wave constant current impulses of duration 0·2 ms) were delivered at 3/s to the median and tibial nerves at the wrist and ankle, respectively. The intensity was adjusted so as to produce a moderate twitch of the appropriate muscles. Following upper limb stimulation, SEPs were recorded with Ag/AgCl disc electrodes attached over the supraclavicular fossa (for N9 potentials), the Cv7 (for N13 potentials) and the hand area of the contralateral sensory cortex, i.e., 7 cm lateral to a point 2 cm posterior to the vertex (for N20 potentials). Following lower limb stimulation the recording sites were over the midline foot area of the sensory cortex, i.e., 2 cm posterior to the vertex (for P40 potentials). All electrodes were referred to a site on the midline 12 cm above the nasion (Fz, International 10–20 System). The input signals were fed into the amplifier system using a band width of 0·8 Hz to 1·6 kHz. Two groups of 512 responses were averaged, with a duration of 32 ms and 64 ms for potentials following median and posterior tibial nerve stimulation, respectively. The abnormalities were classified according to Yu and Jones (1985) in their work on cervical spondylosis: (1) abnormality of N9 ('distal' type) indicated a lesion at or distal to the dorsal root ganglia; (2) abnormality of N13 with normal N9 ('proximal' type) indicated a lesion proximal to the dorsal root ganglia, involving root fibres or the spinal cord; (3) abnormality of N20 with normal N9 and N13, and/or of P40 indicated a spinal cord lesion ('spinal cord' type).

The recordings were made before conservative or surgical treatment and at least 4 months after treatment, and the values compared with control values of the same sex and age groups (*see* Appendix). The control group comprised 10 males and 10 females aged 52 to 78 yrs (mean 66 yrs). These were volunteers who had no neurological symptoms or signs, or diseases which affected the nervous system. Limits of normal latency were defined by the mean ±2.5 SD and amplitude limits by the absolute range of the control groups. Degradation of waveform was also considered an abnormality.

RESULTS

The clinical features, SEPs and radiological findings are summarized in Tables 1 and 2, respectively.

Clinical features (Table 1)

There were 5 males and 1 female and the mean age at presentation was 67 yrs (range 54–76 yrs). The duration between onset and presentation ranged from 1 to 60 months. In only 1 patient (Case 3) was there a history of trauma: she fell from a height of 2 m and landed on her buttocks; this was followed by clumsiness in the hands and weakness in the legs. Disability was moderate in 5 and marked in 1. Cord signs extended above C5 segment in all cases, and consisted of spastic tetraparesis, the legs being more affected and impairment of spinothalamic and posterior column sensation in varying degree. Signs of 'myelopathy hand' were evident: impaired 10 s test in all and positive finger escape sign in 3. Sphincter disturbance was prominent in 3 patients but anterior horn cell damage was not present in any. The signs were asymmetric in 2 patients.

Posterior laminectomy was performed in 2 patients and laminoplasty in 1. These

TABLE 1 CLINICAL FEATURES AND SOMATOSENSORY EVOKED POTENTIALS

			Signs							*Pretreatment SEPs*				*Posttreatment SEPs*			
				Anterolateral column						*Median*		*Tibial*		*Median*		*Tibial*	
Case	*Sex, age (yrs)*	*Duration (mo) and disability*	*Cord level*	*PT*	*SPH*	*ST*	*Posterior column*	*Treatment*	*Follow-up (mo) and response*	*R*	*L*	*R*	*L*	*R*	*L*	*R*	*L*
1(L.H.M.)	M54	2, **	>C5	RL	Pres.	RL	RL	C3–6 lam.	24, ++	PR a	PR a	SP a	SP a	↑	↑	↑	↑
2(C.S.L.)	M66	6, **	>C5	R>L	Pres	RL	R	C3–6 lam.	14, +	PR abs.	PR w,a	SP l	SP l	↑	↑	↑	↔
3(A.Y H)	F68	1, ***	>C5	RL	Pres	RL	RL	C3–6 lam.	6, ++	PR abs	PR w,a	SP abs.	SP w,a	↑	↑	↑	↑
4(C.C.P.)	M63	60, **	>C5	RL	Abs	RL	RL	C4–6 spondyl	40, +	PR l	PR l	N	N	↑	↑	↔	↔
5(C S T.)	M76	24, **	>C5	RL	Abs.	R>L	RL	Con	20, —	PR abs.	PR abs	SP abs	SP abs	↔	↔	↔	↔
6(L.X Z)	M75	24, **	>C5	RL	Abs.	L	RL	Con	6, 0	PR l	N	N	N	ND			

a = reduced amplitude; Abs. = absent; Con. = conservative treatment; F = female; L = left, l = prolonged latency; lam. = laminectomy/laminoplasty; N = normal; ND = not done; PR = 'proximal' type of defect; Pres. = present; PT = pyramidal tract; R = right; SP = 'spinal cord' type of defect; SPH = sphincter disturbance; spondyl. = spondylectomy; ST = spinothalamic tract; w = degraded waveform; > = above or worse; ** = moderate disability; *** = severe disability; + = slight improvement; ++ = marked improvement; — = slight deterioration; 0 = no change on clinical assessment; ↑ = improvement; ↔ = no significant change in SEPs.

patients had continuous and extensive OPLL. Anterior operative decompression by partial spondylectomy, discectomy and fusion was carried out in 1 patient in whom the OPLL was more localized (at C5/6 level). Improvement was marked in 2 and slight in 2. Conservative treatment in the form of a neck collar was given to 2 patients: Case 5, who declined the offer of surgery and whose condition deteriorated gradually over 20 months, and Case 6, whose symptoms were mild and remained so after 6 months.

Radiological findings (*Table 2*)

Plain radiography was informative, diagnosing accurately the presence and extent of the OPLL as confirmed by CT. As OPLL is mostly centrally located, mAPD reflected acquired narrowing of the cervical spinal canal. In all patients, mAPD was at or below the critical value of 10 mm for cervical cord compression. The cervical canals were congenitally narrow, however, in only 2 patients. Spondylosis ranged from mild to marked and in 1 patient (Case 4), posterior osteophytes were present and contributed to cord compression.

Metrizamide myelography was performed in 5 patients and showed thecal and cord compression mainly at the anterior aspect. Cord involvement at multiple levels was shown in 2 patients, and at a single level in 2. In 1 patient, complete block was present at C4/5 level and hence the degree of cord compression at C2/3 and C3/4 levels could not be assessed.

CT showed the site and extent of the OPLL clearly. Significant spondylotic lesions were detected: protruded discs in Cases 1 and 2 and posterior osteophytes in Case 4. The compressive effect of OPLL on the cord was demonstrated clearly in computer-assisted myelography, but could only be inferred in plain CT.

Somatosensory evoked potentials (*Table 1*)

SEPs recorded before surgical or medical treatment showed evidence of posterior column pathway involvement in all 6 patients. Of the 24 nerves stimulated, the 'proximal' type of abnormality was elicited in 11 and the 'spinal cord' type in 8; no abnormality was found in 5. Degradation of waveform, and/or amplitude reduction were the predominant findings in 14 nerves, in 7 of which N13, N20 or P40 were unrecordable. Prolongation of latency, usually of a mild degree, was present in 5 nerves. Recording following treatment was performed in 5 patients. In 4 patients, improvement of the abnormal preoperative SEPs corresponded to the generally favourable response to surgery. In Case 5, the N13, N20 and P40 potentials were already unrecordable at presentation and repeat recording when further deterioration of clinical state took place showed the same findings.

Illustrative case

Case 2. A 66-yr-old dockyard labourer presented with a 6-month history of progressive leg weakness, worse on the right. He sustained several falls and had to use a walking stick 2 months before admission. He also experienced clumsiness of the right hand, paraesthesiae down the right upper extremity and urgency of micturition. There had been no neck symptoms or injury.

TABLE 2. RADIOLOGICAL FINDINGS

	Plain x-ray				*Myelogram*			*CT (Plain or CAM)*				
					Cord compression				*Cord compression*			
Case	*OPLL*	*mAPD (mm)*	*Canal**	*CS*	*Level*	*Anterior*	*Posterior*	*OPLL mAPD (mm)*	*Level*	*Anterior*	*Posterior*	*OPLL/CS/both*
1	Cont	10.1	Ad.	+	4/5	+++	+	2/3–5/6	3/4	++		OPLL
	2/3–5/6	4/5				(Complete block)		(dc at 4/5)	4/5	+++	+	both
					5/6	+		10·5, 4/5	5/6	+		OPLL
2	Cont.	5 7	Ad	++	2/3	++		C2–7	2/3	++		OPLL
	C2–7	4/5			3/4	++		(dc at 3/4)	3/4	++		both
					4/5	+++	+	5.5, 4/5	4/5	+++	+	OPLL
									5/6	+		OPLL
3	Cont.	8.2	Ad.	+	3/4	+++	+	C3–6	Not certain in plain CT			
	C3–6	3/4			4/5	++	+	7.5, 3/4				
					5/6	++	+					
4	Cont	10	na.	+++	5/6	+++	+	C3–7	Nor certain in plain CT			
	C3–7	4/5–6/7						(os at 5/6				
								and 6/7)				
								10, 5/6				
5	Seg.	9.2	na	+	4/5	+++		Lower C4	4/5	+++	+	Both
	lower C4	4/5						and C5				
	and C5							9.0, 4/5				
6	Cont.	5.8	Ad.	++		ND		C2–6/7	Not certain in plain CT			
	1/2–6/7	3/4						6.5, 3/4				

* Canal refers to the sagittal diameter of the cervical spinal canal without OPLL.

Ad. = adequate; CAM = computer-assisted myelography; CS = cervical spondylosis; Cont. = continuous type; dc = disc protrusion, na. = narrow; mAPD = minimal sagittal diameter; os = osteophytes; Seg. = segmental type; + = mild; ++ = moderate; +++ = marked

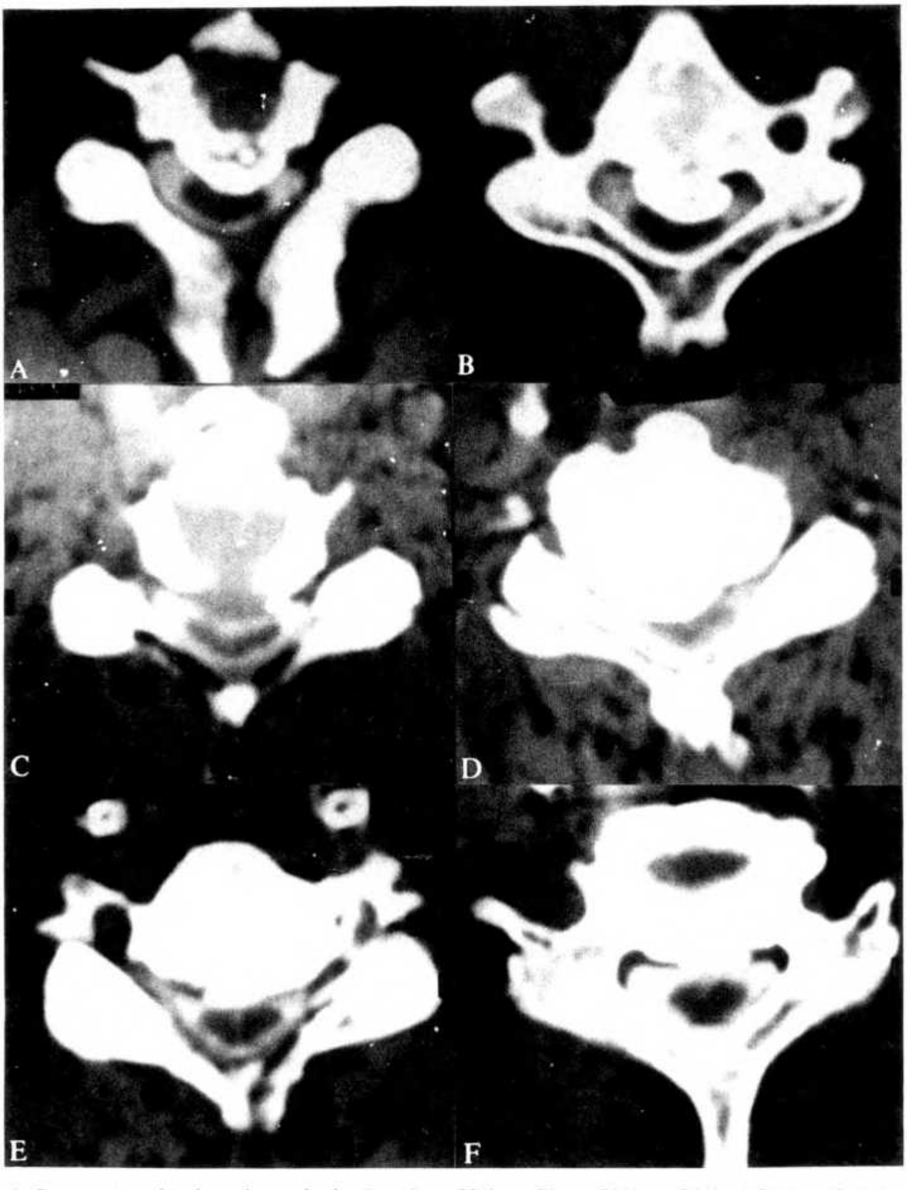

FIG. 1. Computer-assisted myelography in *Case 2*. A, C2/3; B, C3; C, C3/4; D, C4/5; E, C5/6; F, C6/7 levels. At all levels except C3/4, a large-sized OPLL was seen encroaching the spinal canal. At C3/4 level, a central disc protrusion was the culprit. The thecal sac and spinal cord were compressed anteriorly from C2/3 to C5/6 level, most severely from C3 to C4/5.

Examination revealed a full range of neck movement. In the upper limbs there was hypertonia and hyperreflexia. In the right hand, fine movements were impaired, the finger escape sign was positive and grip and release was slow (12 times in 10 s). In the lower limbs there was a moderate spastic paraparesis. Pin prick sensation was reduced in the hands and vibration sense in the right hand and foot.

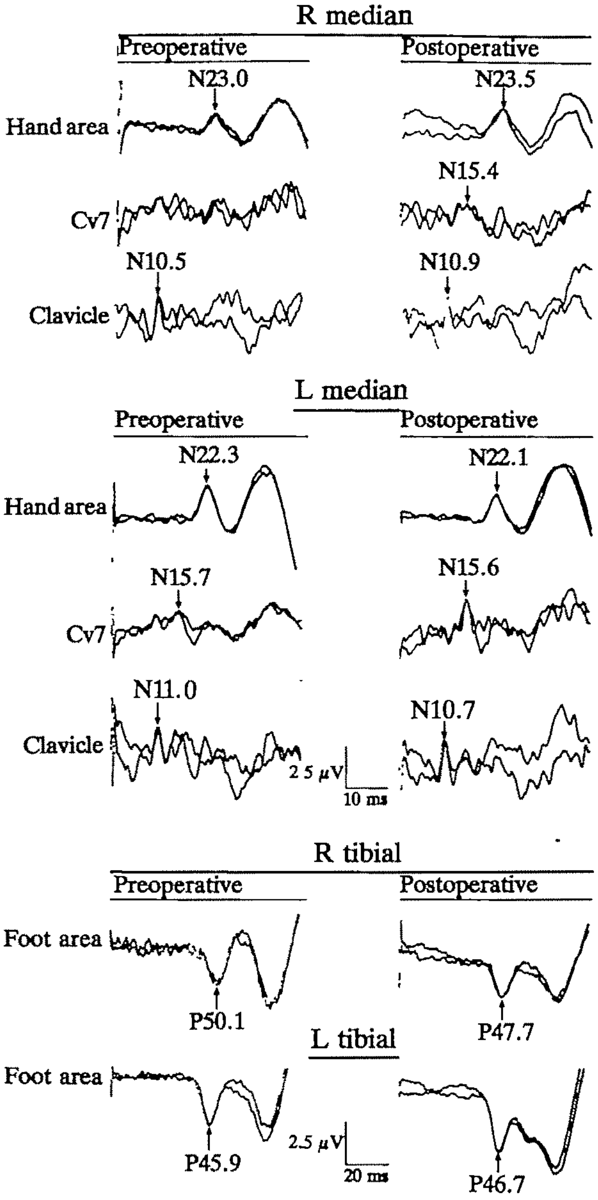

FIG. 2. Preoperative and postoperative SEPs in *Case 2* Note the degraded N13 potentials which improved after surgery. The P40 potentials were delayed bilaterally more so on the right. Postoperatively, the latency of P40 on the right side was considerably shortened, by 2.4 ms, while that on the left remained relatively unchanged.

Cervical myelopathy was diagnosed. Radiographs of the cervical spine showed moderate spondylosis and a continuous OPLL from C2 to C7, narrowing the canal APD by over 60% at C4/5 level. Metrizamide myelography revealed anterior thecal and cord compression at multiple levels, particularly marked at C3/4 and C4/5. Computer-assisted myelography (fig. 1) showed marked

OPLL extending from C2 to C7 vertebral body causing severe cord compression from C3 to C4/5 levels, with significant contribution from a central disc protrusion at C3/4.

A C3 to C7 canal-expansive laminoplasty was performed, resulting in mild improvement, sustained up to the latest follow-up at 20 months. Fine movements in the right hand were slightly better, with faster grip and release (16 times in 10 s) but a persistent finger escape sign. There was less spastic weakness in the legs but he still required a walking stick. Urgency of micturition became less of a problem. Vibration sense in the right hand and foot returned, but the spinothalamic disturbance in the hands remained unchanged.

SEPs (fig. 2) were recorded preoperatively and 6 months postoperatively. N13 potentials were absent on the right, and degraded with reduced amplitude and slightly delayed latency on the left; these abnormalities improved significantly in the postoperative recording. P40 potentials were delayed bilaterally, more so on the right. After surgery the latency of P40 on the right was shortened by 2.4 ms while that on the left remained relatively unchanged. The SEPs therefore correlated with the clinical signs of more severe right-sided posterior column involvement, and reflected the mild clinical improvement following surgery.

DISCUSSION

The incidence of radiological OPLL has been investigated in Japan and South-East Asian countries including Hong Kong (Investigation Committee on OPLL, 1981). This is about 2% in Japan and 1.8% in Hong Kong. The rarity of reports of symptomatic OPLL in Chinese communities is therefore surprising but is probably due to a lack of awareness of this entity. This was the experience in Japan before 1960, but when radiographs of cervical spine between 1934 and 1945 were examined retrospectively, a similar incidence of 2.4% was noted (Investigation Committee on OPLL, 1981). When this condition is more widely recognized, the incidence of symptomatic OPLL in Asian countries is expected to be similar to Japan and considerably higher than in Caucasians.

The clinical picture in this series is similar to other reported cases, with a cervical cord lesion as the predominant feature. The age of onset of symptoms is in or above the sixth decade. The onset is insidious except in those with neck trauma. Neck pain was common in the Japanese series (Ono *et al.*, 1977; Investigation Committee on OPLL, 1981), but was not present in our patients. Spastic tetraparesis of different degrees is invariably present, but signs suggesting anterior horn cell damage are seldom found. Of note are the signs of 'myelopathy hand'. While clumsiness of the hand is one of the commonest complaints in cervical cord lesions, little had been published on the characteristic dysfunction of the hands or fingers until the report of Ono *et al.* (1987). These signs prove to be sensitive for early detection of upper motor neuron lesions in cervical myelopathy and enable quantification of the deficits. Sphincter dysfunction tends to occur only in more advanced cases whereas sensory disturbances, spinothalamic or posterior column, are common. Thus there are no particular features distinguishing OPLL from cervical spondylotic myelopathy (CSM), the commonest cervical cord lesion after middle age (Brain *et al.*, 1952; Clarke and Robinson, 1956). Moreover, as cervical spondylosis is common radiologically above the age of 40 yrs, CSM is often considered as the cause of a cervical cord lesion. The diagnostic clue for OPLL myelopathy lies in careful inspection of the lateral radiographs for a dense ossified strip along the posterior margin of the cervical vertebral bodies (Ono *et al.*, 1977).

The role of CT in CSM has recently been defined (Yu *et al.*, 1986*a*) and this would also apply to OPLL. While CT is not essential for establishing the diagnosis, its cross-sectional image display, high resolution and the use of intrathecal contrast enable a better assessment of the site, size and height of the OPLL, the sagittal diameter of the narrowed spinal canal, as well as the degree of cord compression. Yamamoto *et al.* (1979) evaluated 21 patients with CT and noted a correlation between the shape and extent of OPLL and the 5 patterns of myelopathy as described by Crandall and Batzdorf (1966). Such a correlation is analogous to that recently reported in CSM (Yu *et al.*, 1986*b*).

Detailed studies on SEPs in cervical spondylotic myelopathy have been published (El Negamy and Sedgwick, 1979; Yu and Jones, 1985), but as far as we are aware, this is the first report on OPLL with an SEP study. All 6 patients had abnormal SEPs typical of cervical myelopathy. The 'proximal' type of deficit was more frequently encountered than the 'spinal cord' type. None showed the 'distal' type of abnormality, which is consistent with the observation that OPLL, by virtue of its central location, causes less radiculopathy than cervical spondylosis. Degradation of waveform and reduction in amplitude were more prominent abnormalities than prolongation of latency, which was usually of a mild degree. These findings correlated with posterior column deficits elicited clinically and are characteristic of compressive cervical myelopathy. The abnormalities are therefore similar to CSM (Yu and Jones, 1985), and differ from multiple sclerosis in which marked delays with relative preservation of amplitude and waveform are often seen. As subclinical cord damage in cervical spondylosis can be demonstrated by SEPs (Yu and Jones, 1985), it would be of interest to study SEPs serially in subjects with asymptomatic OPLL or those with mild cord signs in order to ascertain the incidence of subclinical posterior column lesions and the likelihood of progression. The positive correlation between the postoperative clinical state and SEPs in 4 patients suggests that SEPs may be helpful in monitoring progress or deterioration following treatment. However, the patient numbers are small and further prospective studies are required to evaluate the value of SEPs in this aspect.

Cord pathology in OPLL has been reported in a small number of Japanese patients (Tsukimoto, 1960; Ono *et al.*, 1977; Murakami *et al.*, 1978; Investigation Committee on OPLL, 1981). Macroscopically the cord is indented and flattened from anterior compression. Microscopically, severe infarction of the grey matter, ascending demyelination in the posterior columns, descending demyelination in the lateral columns and proliferation of hyalinized small blood vessels are present. These changes are similar to those of CSM (Mair and Druckman, 1953; Payne and Spillane, 1957; Wilkinson, 1960; Ogino *et al.*, 1983). In contrast to the extensive investigations on the still uncertain aetiology and pathogenesis of ligamentous ossification (Okamoto and Yasuma, 1967; Investigation Committee on OPLL, 1981; Motegi *et al.*, 1982), relatively little consideration has been given to the pathogenetic mechanisms of cord damage in OPLL. In this respect, analogy with CSM is useful.

Compression by the ossified ligament is probably the most important factor. Ono *et al.* (1977) stated that 'symptoms were seen most often in those cases where the OPLL occupied more than 60% of the sagittal diameter of the cervical canal', while Nose *et al.* (1987), in a recent study of 74 patients, concluded that 30% stenosis of the sagittal diameter of the canal was critical for the production of myelopathy. Hanai *et al.* (1977) found that in severe myelopathy, the area ratio of the OPLL to the spinal canal in the transverse plane was more than 30%. In earlier reports, reference was not made to the congenital size of the spinal canal, although recently Sato *et al.* (1977) suggested that patients with OPLL usually have a narrow canal as a basic abnormality, especially in those with symptoms. In 2 of our patients, the canal was narrow developmentally and probably contributed to cord damage. However, compared with CSM in which the congenital size of the canal figures more prominently in the pathogenesis than compression by spondylotic protrusion (Mair and Druckman, 1953; Payne and Spillane, 1957; Wilkinson, 1960; Hinck and Sachdev, 1966; Nurick, 1972; Yu *et al.*, 1986*b*), compression by the ossified ligament in OPLL plays a dominant role, probably by virtue of the size it more often attains. Associated spondylotic changes are also an aggravating factor (Shibasaki and Nagamatsu, 1968; Palacios *et al.* 1971; Nakanishi *et al.*, 1974), as is evident in half of our patients.

As a number of patients develop severe disability in spite of a relatively slight narrowing ratio of the spinal canal, mechanical factors other than compression by OPLL are operative (Investigation Committee on OPLL, 1981). Movements of the cervical spine may play a role as in CSM. O'Connell (1956) suggested that in CSM cases where spondylotic protrusions were not severe, repeated cord trauma through rubbing of the cord against the bony ridge during neck flexion resulted in myelopathy. Adams and Logue (1971) documented increased cervical spine movement in patients with myelopathy, and also noted a less favourable response to laminectomy in myelopathy patients with a high range of movement. However, because the OPLL tends to immobilize and maintain the spine in a relatively fixed position, the dynamic factor may not contribute as much as in CSM. Acute trauma may precipitate or aggravate cord damage in cervical spondylosis (Brain *et al.*, 1952; Symonds, 1953) or ankylosing spondylitis (Foo *et al.*, 1985), and probably also in OPLL as in 1 of our patients. Vertebral subluxation, which occurs in about a quarter of CSM cases (Yu *et al.*, 1986*a*) and may contribute to cord damage (Payne and Spillane, 1957), has seldom been observed, again probably due to the immobilizing effect of the ossified ligament.

The histological changes of the cord in both OPLL and CSM are similar to those of ischaemia consequent upon reduction of blood supply in the distal distribution of the anterior spinal artery (Mair and Druckman, 1953; Tsukimoto, 1960; Ono *et al.*, 1977; Murakami *et al.* 1978). The level of vascular occlusion is debated but it is likely that impaired microcirculation leads to ischaemic cord damage. This view is supported by the findings of Gooding *et al.* (1976), who documented reduced blood flow at the compression site of canine cervical cords following the application of moderate compression. Brodkey *et al.* (1972) also concluded that ischaemia played an important part in conduction block caused by spinal cord compression in cats.

In summary, the pathogenetic mechanisms of OPLL myelopathy are therefore multiple, with compression by the ossified ligament as the most important factor. A congenitally narrow canal, spondylosis and trauma are probably aggravating factors. Movement of the cervical spine resulting in friction between the cord and OPLL could contribute and would explain cases without marked canal narrowing from OPLL. Pathological and experimental evidence suggests that cord damage is probably mediated by ischaemia due to disturbances of the microcirculation.

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APPENDIX

CONTROL VALUES OF N13, N20 AND P40 SOMATOSENSORY EVOKED POTENTIALS IN 10 CHINESE MALES AND 10 FEMALES AGED 52 TO 78 YRS

	Male	*Female*
Mean body height (cm)	160	152
N13 latency (ms)		
(mean, SD, upper limit)		
Right	13.4, 0.8, 15.4	12.4, 0.6, 13.9
Left	13.6, 0.8, 15.1	12.4, 0.5, 13.7
N13 amplitude (μV)		
(lower limit)		
Right	−2.1	−2.6
Left	−1.3	−2.1
N20 latency (ms)		
(mean, SD, upper limit)		
Right	19.2, 1.1, 22.0	18.0, 0.9, 20.3
Left	19.2, 1.0, 21.7	18.1, 0.5, 19.3
N20 amplitude (μV)		
(lower limit)		
Right	−2.7	−2.7
Left	−2.3	−1.4
P40 latency (ms)		
(mean, SD, upper limit)		
Right	39.4, 2.0, 44.4	38.8, 2.0, 43.8
Left	39.5, 2.2, 44.9	38.0, 2.2, 44.3
P40 amplitude (μV)		
(lower limit)		
Right	0.6	0.5
Left	0.8	0.4

Brain, (1988), **111**, 785–799

GAMMA-AMINOBUTYRIC ACID CONCENTRATION IN BRAIN TISSUE AT TWO STAGES OF ALZHEIMER'S DISEASE

by S. L. LOWE, P. T. FRANCIS, A. W. PROCTER[1], A. M. PALMER, A. N. DAVISON AND D. M. BOWEN

(From the Miriam Marks Department of Neurochemistry, Institute of Neurology, Queen Square, and [1]Department of Psychiatry, UMDS-Guy's Hospital, London)

SUMMARY

The concentration of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), was measured in the cerebral cortex obtained at diagnostic craniotomy from 10 patients with Alzheimer's disease of 3 yrs mean duration and 6 patients with other causes of dementia, and from 31 subjects undergoing other neurosurgical procedures (for which removal of apparently normal tissue was necessary). GABA content of 5 areas of the cerebral cortex and the cerebellar cortex was measured postmortem in the brains of 23 Alzheimer and 19 control subjects and 5 patients with other causes of dementia. Fourteen of these specimens, including 7 from patients with Alzheimer's disease of 8 yrs mean duration, were obtained within 3 h of death. These were processed in a similar manner to the neurosurgical specimens and are regarded also as fresh tissue samples. The remaining 33 specimens are regarded as conventional postmortem samples as the mean interval of death to autopsy was 21 h.

GABA concentration in conventional autopsy specimens from Alzheimer subjects was not reduced as compared with controls in either cingulate or cerebellar cortex. In the inferior parietal cortex, agonal status confounded this comparison. The concentration was reduced in superior parietal, frontal and temporal cortex but there is a possibility that agonal state also confounded these comparisons. There was no deficit in GABA concentration in fresh cortical tissue from Alzheimer patients except for the temporal lobe from autopsy specimens. The content of somatostatin-like immunoreactivity was, like GABA, found to be comparable to control in some groups of Alzheimer specimens. It is argued that the deficits in autopsy samples and lack of change in surgical specimens is likely to be due to the duration of illness at the time of sampling. Losses of choline acetyltransferase activity were observed in all groups of Alzheimer specimens in all areas of brain studied. The data are consistent with other results which suggest that cholinergic under-activity is most closely related to the clinical course of Alzheimer's disease.

INTRODUCTION

The discovery of reduced choline acetyltransferase (ChAT) activity in Alzheimer's disease (AD) led to the hypothesis that AD is a disorder of acetylcholine deficiency. Crucial evidence in support of this hypothesis would be the demonstration that other neurotransmitters are unaffected, including inhibitory gamma-aminobutyric

Correspondence to: Dr D. M Bowen, Department of Neurochemistry, Institute of Neurology, 1 Wakefield Street, London WC1N 1PJ, UK.

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acid (GABA, Bowen *et al.*, 1976*a*). Yet both markers of these neurotransmitters (glutamic acid decarboxylase and ChAT activities) were reduced by at least 45% in AD samples. However, detailed studies of the brains of humans and experimental animals (Bowen *et al.*, 1976*a*, *b*, 1977; Spillane *et al.*, 1977) suggested that loss of decarboxylase activity is secondary to interaction between the way in which the AD patients died and pathophysiology (exemplified by reduced cerebral blood flow and perhaps a defective respiratory index; *see also* Spokes *et al.*, 1979).

Many interneurons in the cerebral cortex use GABA as neurotransmitter and, probably, a small proportion, somatostatin (Jones, 1986). The concentrations of these substances have therefore been used as an index of interneurons in AD. There is now evidence of reduced GABA concentration postmortem in the temporal, frontal and occipital lobes (Rossor *et al.*, 1982; Ellison *et al.*, 1986; Sasaki *et al.*, 1986). The reductions found in some studies (Rossor *et al.*, 1982; Sasaki *et al.*, 1986) are less substantial and widespread than those reported by Ellison *et al.* (1986) who assayed subjects of similar age but included only pathologically severe examples of the disease. Beal *et al.* (1986) and Davies *et al.* (1980, 1982), who also studied only pathologically severe subjects, both found greater losses of somatostatin-like immunoreactivity (SLIR) than those reported by others (Rossor *et al.*, 1980; Ferrier *et al.*, 1983; Reinikainen *et al.*, 1987; Tamminga *et al.*, 1987). Some of the reported data show loss of at least 45% of the SLIR, even in studies where subject selection criteria were not described (Rossor *et al.*, 1980; Tamminga *et al.*, 1987). Losses of neuronal uptake sites for GABA, based on high affinity uptake of labelled transmitter into subfractionated frozen and thawed autopsy tissue, were thought by Hardy *et al.* (1987) to occur throughout the cortex and to be well in excess of 45 %. It has also been argued that neurotransmitter replacement strategies should take loss of somatostatin into consideration (Tamminga *et al.*, 1987). However, tetrahydroaminoacridine, a centrally acting anticholinesterase, has recently been reported to elicit striking improvements in a group of AD patients (Summers *et al.*, 1986). The action of this compound may be complex as it is similar in structure to the aminopyridines, which have been shown to act on potassium and calcium channels and cause increased release of acetylcholine and other neurotransmitters including GABA (*see* Tapia and Sitges, 1982; Gibson and Peterson, 1986).

Siesjö (1978) laid emphasis on the sensitivity of the concentration of GABA to insults affecting energy metabolism. High affinity uptake of GABA is an energy dependent process likely to be affected by altered energy metabolism. A possible disorder of energy metabolism is a feature of AD (Procter *et al.*, 1988*a*; Sims and Bowen, 1987) and animal studies indicate that GABA content changes postmortem (*see* Rossor *et al.*, 1982). Changes perimortem, such as terminal coma, probably affect metabolic and postmortem changes in an unpredictable manner (Bowen *et al.*, 1977). Thus complex epiphenomena may have influenced postmortem findings. To attempt to address this issue, the GABA content has been determined of samples from subjects where the duration of terminal coma was known and the autopsy examination was promptly performed. Subjects were not deliberately selected and the samples were obtained some 8 yrs after emergence of symptoms. Another series has been assayed which was obtained at diagnostic craniotomy, 3 yrs after onset. This has been used to establish whether GABA content is reduced at the early to middle stage of the disease. It was thought that such data would have a bearing on the issue of whether the inclusion in postmortem studies of only pathologically severe examples of the disease obscures a deficit in a neurotransmitter which might be corrected by replacement strategies.

MATERIALS AND METHODS

Tissue samples

Postmortem examinations were made of patients with dementia and undemented control subjects, matched for age and delay in autopsy (Table 1). The brains were bisected sagittally, one hemisphere was used for histological examination and the other frozen at −70 °C until required for neurochemical studies. Subjects with AD had a clinical diagnosis together with senile plaque formation and neurofibrillary degeneration consistent with a diagnosis of AD (Bowen *et al.*, 1976*a*; Wilcock and Esiri, 1982). There was no selection of cases by disease severity, gender, drug treatment or agonal status and the specimens included 3 and 6 left hemispheres from control and AD subjects, respectively. Clinical and histopathological data indicated that the control group was free of gross psychiatric or neurological disturbance.

The temperature of hemispheres was subsequently raised to −10 °C and 0.5 cm coronal slices were cut. Cerebellar cortex and blocks of grey matter with underlying white matter were removed from the region of the following Brodmann areas (BA) of the cerebral cortex; BA 21 pooled with 22 (temporal cortex), BA 40 (inferior parietal cortex), BA 7 (superior parietal cortex), BA 23 (cingulate cortex) and BA 9 (frontal cortex). The blocks were maintained at −70 °C until they were thawed (0–2 °C) and the grey matter (containing all cortical layers) dissected free of meninges and white matter. Aliquots were refrozen and maintained (−70 °C) for later determination of GABA content and ChAT activity. The samples were matched for storage time.

Postmortem examinations were also made of 12 other patients who had a clinical diagnosis of dementia and 2 undemented control subjects, within 3 h of death (Table 1). The brains were removed and bisected sagittally. One hemisphere was fixed in formalin for histological examination and the other sliced coronally into 1 cm thick slices which were placed into an ice-cold modified Krebs-Ringer phosphate buffer containing glucose (physiological buffer) and transferred to the biochemistry laboratory in an insulated box as previously described (Bowen *et al.*, 1982*a*). Seven demented subjects had AD diagnosed as above and the remainder were classified as demented controls. One of these brains (AP1, a 91-yr-old subject) showed severe plaque formation but little or no neurofibrillary degeneration. The other demented controls were diagnosed as having depressive pseudodementia (AP6), multisystem degeneration (AP7), histologically confirmed Pick's disease (AP5) and multiinfarct dementia (AP10). Routinely, within 4 h of death, blocks of grey matter and underlying white matter were removed from the slices in the region of BA 22 (BA 20 for assay of SLIR content, temporal cortex), BA 9 (frontal cortex) and BA 7 (parietal cortex). The grey matter was dissected (on ice) free of meninges and underlying white matter and frozen for later determinations of GABA and SLIR contents, and ChAT activity.

Samples of grey matter and underlying white matter of temporal and frontal lobes were obtained by neurosurgery as previously described (Bowen *et al.*, 1982*b*; Neary *et al.*, 1986; Sims *et al.*, 1983). Control samples were from patients undergoing neurosurgical treatment for tumours (craniopharyngioma, glioma, meningioma, pituitary tumour, epidermoid cyst) or aneurysms for which removal of apparently normal tissue was a necessary part of the surgical procedure. The other samples were

TABLE 1. DEMOGRAPHY OF SUBJECT GROUPS

Samples obtained by	*Age (yrs)*	*Sex**	*Duration of terminal coma (h)*	*Postmortem interval to autopsy (h)*
Conventional autopsy				
AD (16)	81±8, 61–93	6 M	n.d.	21±10, 4–24
Control (17)	78±11, 57–90	10 M	n.d.	21±10, 5–30
Prompt autopsy				
AD AP2**	83	F	72	3.0
AP3	58	F	72	2.0
AP4**	69	F	48	1.5
AP8	80	F	< 1	2.0
AP9	78	F	336	1.75
AP11	60	M	< 1	3.0
AP14	64	F	< 12	2.5
Controls				
SB34	67	M	< 1	1.0
SB37	68	F	< 1	1.0
Demented controls				
AP1**	91	M	240	2.5
AP5	79	F	72	1.5
AP6	72	M	< 1	1.0
AP7	63	F	12	2.0
AP10**	82	F	96	1.5
Neurosurgery				
Frontal lobe				
AD (3)	64±4, 61–64	0 M		
Controls (16)	53±10, 39–69	7 M		
Demented controls (5)	54±5, 46–59	4 M		
Temporal lobe				
AD (7)	60±4, 55–68	4 M		
Control (15)	58±8, 47–68	7 M		
Demented controls (1)	43	1 M		

Values are mean ± SD with range (no. of subjects in parenthesis, except where individual subjects are tabulated). * Where more than 1 subject is described the number of males is given; ** drug-free prompt autopsy, demented subject; n d. = not determined.

removed at diagnostic craniotomy from the nondominant hemisphere of patients with a clinical diagnosis of primary degenerative dementia. Subjects with AD were diagnosed as above and the remainder (demented controls) are thought to have other dementias as the histological examination showed no specific changes (Sims *et al.*, 1983; Neary *et al.*, 1986). Less than 5 min after removal from the brain, samples were placed in ice-cold physiological buffer. After 20–40 min in buffer the tissue was dissected. The outer 2 mm (approximately) of specimens was discarded if diathermy was used, grey matter was dissected as above and representative aliquots were maintained at −196 °C for later determination of GABA content and ChAT activity. Samples from demented and control subjects were matched for age (Table 1) and storage time.

All specimens were obtained under procedures approved by the relevant Hospital Ethical Committees.

Biochemical determinations

Grey matter (100 mg) from postmortem tissue was homogenized in 10 vol of 10 mM sodium phosphate buffer (pH 7.4) using a 1 ml glass-teflon motorized homogenizer assembly (Tri-R, Stir-R Instruments, Rockville Center, NY, USA). In order to extract the free GABA in the tissue, an aliquot (150 μl) of the homogenate was mixed with 9 vol acidified methanol (8.4 ml 0.1 M HCl/100 ml methanol) containing internal standard (alpha-amino adipic acid). The mixture was left for 20 min at 4 °C and centrifuged (15,400 g, 10 min, 4 °C). The concentration of GABA in the supernatant fraction was then determined by high performance liquid chromatography (HPLC). The apparatus consisted of a gradient elution system (Model 300/02; Applied Chromatography Systems, Macclesfield, Cheshire, UK, linked to a microcomputer, Trilab 2000, Trivector Scientific Ltd., Sandy, Beds, UK), injection valve with 20 μl loop (Model 7125 Rheodyne Inc., Cotati, CA, USA), a reverse phase octadecyl polymer-coated silica-column (particle size 3 μm, 100 by 4.6 mm inner diameter; Techsphere 3, HPLC Technology) together with a fluorometric detector (Fluoromonitor III, LDC, Stone, Staffs, UK; excitation 370 nM filter, emission, 418–700 nM wavelength band pass). The eluant was a mixture of 0.05 M sodium phosphate buffer (pH 5.7) and methanol in the proportion (v/v) 80:20 for solvent A and 20·80 for solvent B. A multilinear 45 min gradient was used 85% A, changing to 15% A over 35 min, returning to 85% A. Precolumn o-phthaldialdehyde/2-mercaptoethanol derivitization of amino acids was carried out as described by Joseph and Davies (1983). Peak areas were quantified with a microcomputer (Trilab 2000), and peak identity and purity established by elevating peak areas by addition of authentic standards and by comparing retention times with standards under different chromatographic conditions. Concentrations were determined by calibration with authentic standards and correction of internal standards.

Grey matter (20–50 mg) from tissue removed at neurosurgery was analysed for GABA content as described above, with minor modifications (Procter *et al.*, 1988*b*)

ChAT activity and protein and SLIR content of unfractionated cell free homogenates were determined as previously described (Sims *et al.*, 1983; Francis *et al.*, 1987*a*). All values are expressed relative to the concentration of protein.

Statistical methods

Results are expressed as mean ± SD. Group means were compared using the 2-tailed Students t test with untransformed or log transformed data as appropriate (Fisher F test). Intercorrelation of variables was tested by the 2-tailed Spearman rank correlation (r_s).

RESULTS

P 35,573

Influence of antemortem and postmortem variables

The duration of illness of the patients studied by biopsy was shorter than those at prompt autopsy (2.7 ± 1.7 yrs, range 1–5, and 8.4 ± 5.7 yrs, range 3–17, respectively; $P < 0.01$).

The neurochemical values showed few associations with either age or postmortem interval to autopsy. There were declines in GABA concentration of control samples obtained by conventional autopsy with age in frontal cortex ($r_s = -0.51$, $P < 0.05$) and with postmortem delay in temporal cortex ($r_s = -0.54$, $P < 0.05$).

The effect of various factors on the neurochemical data other than those for which the AD and control samples were matched, were assessed by dividing subjects obtained by neurosurgery or conventional autopsy into appropriate subgroups. None of the measures in either control or AD tissue samples were related ($P > 0.05$) to the patients' gender or the hemisphere assayed.

The GABA content of samples may be influenced by agonal state as the value (nmol/mg protein, units) for the inferior parietal cortex of the 7 controls with protracted illness was 17.2 ± 4.2 units which was reduced ($P < 0.02$) to 74% of the value for the 9 controls of sudden death (23.3 ± 4.6 units). No other differences with agonal state were found, which conforms with previous reports of a general lack of agonal effects (*see* Rossor *et al.*, 1982). However, most of the AD subjects had a protracted illness and the GABA content of inferior parietal cortex (15.1 ± 3.2 units, n = 13) was not reduced compared with the controls (above) of similar agonal state (which were of comparable age and postmortem delay). This was also true in all regions studied so there is a possibility that agonal state confounds all postmortem comparisons of GABA values.

Of the patients with AD (conventional autopsies), 60% had received neurotropic drugs (usually phenothiazines and benzodiazepines) but the GABA content in drug treated subjects was not significantly different ($P > 0.05$) from those of subjects free of medication for at least 1 wk before death. The variability in the GABA content of samples obtained by prompt autopsy (fig.) was apparently unrelated to drug treatment, duration of coma, age or postmortem interval to autopsy. Patients undergoing diagnostic craniotomy were drug free for at least 1 wk before surgery. The GABA content of temporal cortex from AD patients free of medication for at least 1 yr (18 and 25 units) was similar to that of the other AD patients who had received neurotropic drugs (mean 20, range 17–22 units); the drug-free AD patients sampled from the frontal lobe had 22 and 23 units (fig.).

Control neurosurgical tissue was usually obtained from procedures carried out either to gain access to a tumour or for associated decompression of the skull. When adjacent samples were examined histologically, no obvious abnormalities were seen, but the presence of abnormal tissue or changes due to compression damage in some samples cannot be completely excluded. Comparison of results for samples of each lobe separated into 2 groups according to the associated tumour types (craniopharyngiomas and tumours of the temporal lobe, and meningiomas and other tumours of the frontal lobe) did not reveal significant differences ($P > 0.05$) in the concentration of GABA. The overall results have therefore been used as an indication of values for normal tissue.

Early to middle stage of AD

The concentration of GABA in the temporal cortex obtained by neurosurgery from patients with AD was not significantly different from controls (*see* fig.), whereas the concentration was increased to 145% of control in the frontal cortex. The 2 highest GABA values of the biopsy samples from the temporal lobe (fig.) were from the patients with the shortest duration of illness (1 yr). The group of demented controls obtained at diagnostic craniotomy had a GABA content similar to control in both lobes. Table 2 shows the ChAT activity of samples assayed for the concentration of GABA. Four of the AD samples of temporal cortex obtained by neurosurgery were assayed for ChAT (Table 2) which provides a reliable estimate of the deficit in acetylcholine synthesis and the other 3 (patients F, H and O of Neary *et al.*, 1986) were directly assayed for acetylcholine synthesis. In these 3, the value of 2.5 ± 0.8 dpm/mg protein/min for acetylcholine synthesis is significantly reduced ($P < 0.001$) from the control value of 6.9 ± 1.3 units (n = 13, Neary *et al.*, 1986). Thus in the group of 7 AD biopsy samples of temporal cortex assayed for GABA content (fig.), cholinergic markers are reduced to between 31 and 36% of control with sparing of the GABA content. One AD biopsy sample from the

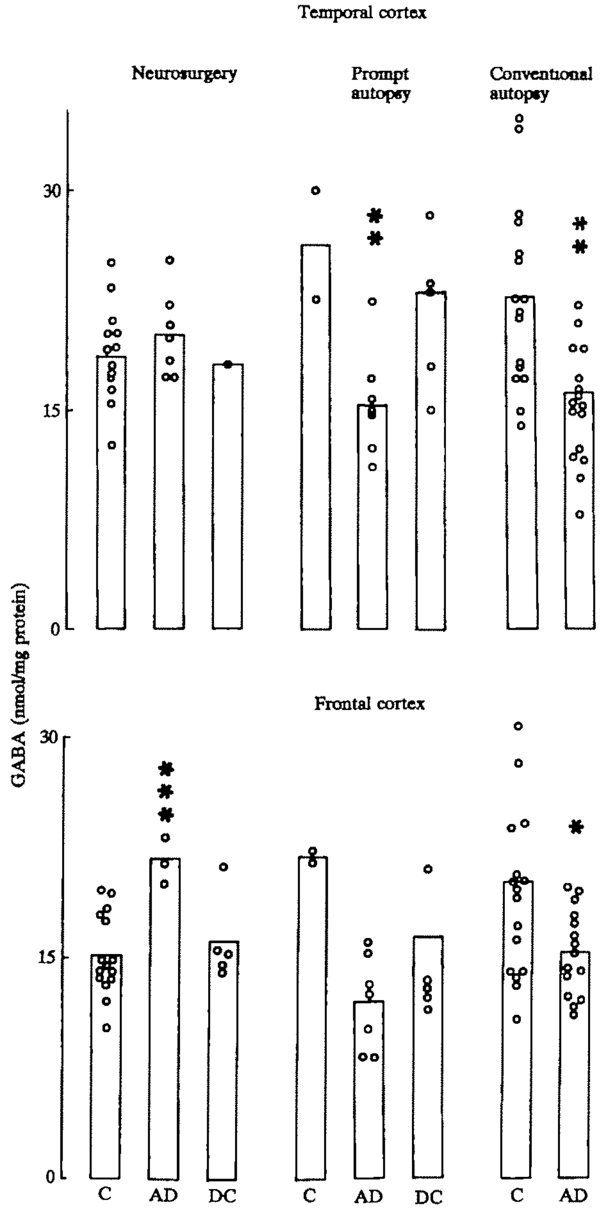

FIG. GABA concentrations of brain samples obtained antemortem (by neurosurgery) and either up to 3 h (prompt autopsy) or 1 day (conventional autopsy) postmortem. After removal from the brain the neurosurgery and prompt autopsy samples were processed by similar methods but the intervals between onset of AD and sampling were different (*see* text). Circles identify values for individual controls (C), demented controls (DC) and Alzheimer's disease (AD). Histobars depict the mean value for each group (except for the temporal cortex of DC which is a single subject) Asterisks identify significant alterations in AD compared with the corresponding control (C and DC by prompt autopsy were pooled for the analysis), $*P < 0.02$, $**P < 0.01$, $***P < 0.001$ by 2-tailed Student's t test.

TABLE 2 ChAT ACTIVITY (pmol/mg protein/min) OF THE CORTEX OF CONTROL AND AD SUBJECTS OBTAINED BY NEUROSURGERY AND PROMPT AND CONVENTIONAL AUTOPSY

Samples obtained by	*Temporal cortex*	*Frontal cortex*
Neurosurgery		
AD	28 ± 14 (4)*	54 ± 38 (3)
Controls	91 ± 34 (6)	103 ± 46 (21)
Demented controls	74	97 ± 29 (4)
Prompt autopsy		
AD	26 ± 11 (7)***	27 ± 18 (7)**
Controls	108, 112	85, 92
Demented controls	122 ± 43 (5)	77 ± 34 (5)
Conventional autopsy		
AD	38 ± 24 (14)***	39 ± 16 (15)***
Controls	113 ± 36 (17)	78 ± 25 (17)

Samples were as in fig. except for the neurosurgical controls (Palmer *et al.*, 1987). One demented control sample of frontal cortex obtained by neurosurgery had an anomalous ChAT activity (38 units) and was excluded from the analysis. Asterisks identify significant alterations in AD compared with the corresponding control (prompt autopsy controls and demented controls were pooled for the analysis). * $P < 0.02$; ** $P < 0.005$; *** $P < 0.001$ by 2-tailed Students t test. Values are mean ± SD (no. of subjects).

frontal lobe had very low ChAT activity (17 pmol/mg protein/min) but not GABA content (22 units, fig.).

Late stage of AD

In samples obtained from AD subjects some 24 h after death, GABA concentration was reduced to 71, 76 and 79% of control in the temporal, frontal and parietal cortex, respectively, but spared in the cingulate cortex (conventional autopsy, fig., Table 3).

Evidence for a deficit in GABA was also detected with temporal cortex obtained within 3 h of death, the content being reduced in AD to 76% of control (prompt autopsy, fig.). This may slightly underestimate the loss of GABA as the 2 demented control subjects with the lowest GABA content had Pick's disease and multiinfarct dementia. The other demented controls had GABA contents within the range of that of the undemented control subjects. The AD sample with the highest GABA value was from AP8 which had the shortest interval between emergence of symptoms and death (3 yrs).

The concentration of GABA in the frontal cortex obtained by prompt autopsy was relatively high in 2 AD subjects (fig.). These were the older subjects which is consistent with reports that such subjects show less severe and widespread biochemical changes (*see* Rossor and Iversen, 1986). The concentration of GABA in a region usually devoid of histopathological change (cerebellar cortex) from 12 AD samples obtained by conventional autopsy was 21.1 ± 7.4 units which was not significantly different from that of 11 controls (23.5 ± 5.3 units).

TABLE 3. GABA CONCENTRATION (nmol/mg protein) AND ChAT ACTIVITY (pmol/mg protein/min) OF THE CORTEX OF CONTROL AND AD SUBJECTS OBTAINED BY CONVENTIONAL AUTOPSY

	Superior parietal cortex	*Cingulate cortex*
GABA		
Controls	20.3±4.9 (14)	19.3±4.3 (16)
AD	16.0±3.6 (15)*	16.4±4.8 (16)
ChAT		
Controls	59±23 (16)	66±28 (17)
AD	31±17 (15)**	34±16 (16)*

Values are mean±SD (no of subjects). * $P < 0.02$; ** $P < 0.005$ by 2-tailed Student's t test.

TABLE 4 SLIR CONTENT (fmol/mg protein) OF THE CORTEX OF CONTROL AND AD SUBJECTS OBTAINED BY PROMPT AUTOPSY

	Temporal cortex	*Frontal cortex*	*Parietal cortex*
AD (n = 7)	802±261*	1025±384	630±201
Control (n = 7)	1194±258	1145±361	899±269

Prompt autopsy controls and demented controls were pooled for this analysis. ChAT activity of the parietal cortex was 26±11 and 68±30 pmol/mg protein/min ($P < 0.01$) for AD and controls, respectively. * $P < 0.02$ by 2-tailed Student's t test. Values are mean±SD.

ChAT activity was quite uniformly reduced in AD postmortem (Tables 2–4). The concentration of SLIR in the temporal cortex obtained by prompt autopsy was significantly reduced in AD to 67% of control whereas the contents of the frontal and parietal cortex were not reduced (Table 4).

DISCUSSION

Postmortem studies may be biased by unintentional selection of subjects for which institutional care was necessary (Procter and Bowen, 1987). We did not deliberately select cases and the changes in GABA and somatostatin concentration and ChAT activity are generally comparable to those reported in other studies where selection criteria were not described (Table 5). Loss of GABA from the temporal lobe is greater than from all other lobes and the cingulate cortex except for 1 study. Somatostatin content is reduced more than that of GABA but ChAT activity is clearly the most affected. (A possible exception to this is in the inferior areas of the parietal and frontal lobes where the extent of loss of somatostatin seems to be comparable to that of ChAT activity.)

The main finding of this study of neocortical tissue from subjects with AD was that of reduced GABA content in the series obtained postmortem but not antemortem. There is a possibility that differences in agonal status had confounded all comparisons of GABA concentrations in conventional autopsy samples, as the content was not reduced in any region compared with controls of similar agonal

TABLE 5 SUMMARY OF PREVIOUSLY PUBLISHED AND PRESENT DATA FOR GABA AND SOMATOSTATIN CONTENT AND ChAT ACTIVITY (PERCENT OF CONTROL) OF THE CEREBRAL CORTEX OF AD SUBJECTS OBTAINED BY CONVENTIONAL AUTOPSY

	No selection of subjects			*Severe examples of the disease*		
Brain Region	*GABA (Various authors)*	*Somatostatin (Various authors)*	*ChAT (Rossor* et al., *1982, and present series)*	*GABA (Ellison* et al., *1986)*	*Somatostatin (Davies* et al., *1982; Beal* et al., *1986)*	*ChAT (Davies* et al., *1982)*
Temporal cortex						
Brodmann area 20	68, n.s.	n.s.**	41, 38*	55	38, 25	8
21	71*, 72	53, 57, 61	39, 33*	58	25, 35	13
28	69	n.d.	42, 44*	65	21	n.d.
38	n.s.	n.d.	40, 33*	74	38	n.d.
Frontal cortex						
Brodmann area 6	n.s.	64	55, n.d.	73	39	n.d.
9	76* n.s., n.s.	n.s., n.s., n.s.**	49, 50*	72	37	n.d.
10	n.s.	n.s.	57, n.d.	n.d.	50	12
11	63, n.s.	49**	52, 51*	n.d.	50	n.d.
Parietal cortex						
Brodmann area 7	79*, n.s.	55, n.s.	51, 53*	n.d.	n.d.	n.d.
39/40	n.s.	47, n.s.**	n.d., 55*	n.d.	43	10
Cingulate cortex						
Brodmann area 23	n.s.*	n.d.	n.d., 52*	n.d.	n.d.	n.d.
24	n.s.	n.s.	54, 58*	73	55	n.d.
Occipital cortex	77, n.s.	n.s.**	47, 45*	74	22	14

Brodmann areas (BA) are approximate as not all use this terminology. * Identifies present series. ** Study of only 4 AD subjects (Arai *et al.*, 1984), mean values as percent of control are 98 (BA 20), 91 (BA 9), 46 (BA 40) and 86 (occipital). Other authors are: Sasaki *et al.*, 1986; Rossor *et al.*, 1982; Ferrier *et al.*, 1983; Francis *et al.*, 1987*a*; Rossor *et al.*, 1980, Tamminga *et al.*, 1987. ChAT activity of the present series is from either Tables 2 and 3 or A. W. Procter *et al.*, unpublished results. n.s. = not significantly different from control; n.d. = not determined.

status. However, it seems unlikely that differences in sex distribution, drug treatment or hemisphere assayed between groups had influenced GABA concentration and the subjects were matched for age, postmortem delay and duration of terminal coma. The antemortem and other series were not matched for duration of illness so this is most likely to have influenced the results. The difference between surgical and autopsy specimens is, therefore, likely to be due to length of disease rather than any effect of such factors as agonal state and postmortem delay.

The GABA content of temporal cortex was spared (100 and 108% of control, fig.) in the 2 patients biopsied where the interval between emergence of symptoms and surgery was longest (5 yrs). Cholinergic markers were less than 50% of control in these patients (B and P of Neary *et al.*, 1986). The other patients were biopsied between 1 and 3 yrs after onset and there is evidence of GABA values higher than control (fig.) whereas cholinergic markers are consistently reduced. Such markers may be a more sensitive indicator of denervation than the content of GABA (*see* Palmer *et al.*, 1987), but these along with other findings (reviewed later) indicate that cholinergic, but not GABAergic, nerve endings selectively degenerate in the early to middle stage of the disease.

AD is a progressive condition and the data in Table 5 suggest that the GABA content is reduced more in AD subjects who died with severe pathological changes and this also appears to be so for the concentration of SLIR. In prompt (Table 4) and conventional (Francis *et al.*, 1987*a*) autopsy samples, the SLIR content of AD temporal cortex was 61–67% of control. This agrees with Ferrier *et al.* (1983) and Rossor *et al.* (1980), yet in our biopsy series the peptide was not found reduced (Francis *et al.*, 1987*a*). Thus the length of disease as well as a probable related factor (severity of pathological change) seem to determine the concentrations of both GABA and SLIR. Cholecystokinin, neuropeptide Y, vasoactive intestinal peptide, substance P and neurotensin are other neuropeptides that have been measured and have not been found consistently reduced. They (together with GABA and somatostatin) are found in cortical interneurons (Jones, 1986), so these observations indicate that many constituents of interneurons are not selectively affected.

Therapeutic implications

Other neurons in the cortex not examined in the present study show substantial neurofibrillary degeneration postmortem, in particular the pyramidal neurons of the hippocampus and possibly corticofugal neurons (Pearce *et al.*, 1984). Moreover, the distribution of both neurofibrillary degeneration (Pearson *et al.*, 1985) and senile plaques (Rogers and Morrison, 1985) are said to coincide with the corticocortical association tracks. Hauw *et al.* (1986), in addressing apparently related phenomena, attributed the near absence of change in neuronal density of AD subjects in general to 'collapse of the neuropil' of the cerebral cortex brought about by loss of structures organized in a columnar manner. The stages of the disease at which 'collapse' occurs and the hippocampus becomes involved are unknown, but cerebral biopsy samples from AD subjects seem to contain reduced numbers of pyramidal neurons based on cell counts with allowance for reduced cortical thickness (Neary *et al.*, 1986). However, such correction could be inappropriate as it remains to be established that reduced cortical thickness is due to loss of pyramidal neurons. When all sizes of neurons are counted in the cerebral cortex a reduced density is not conspicuous in either biopsy samples (Davies *et al.*, 1987) or in elderly AD subjects postmortem (Mountjoy *et al.*, 1983).

The study of biopsy samples indicates a quite uniform deficit, in AD, of cholinergic nerve endings in the frontal and temporal lobes as evidenced by three markers for these structures: ChAT activity, 'high affinity' uptake of [^{3}H] choline into and the synthesis of acetylcholine by tissue prisms (reviewed by Sims and Bowen, 1987). By contrast, glutamic acid decarboxylase activity as well as GABA content are not reduced. Potassium-stimulated tissue prisms of AD patients release at least as much GABA as controls and a binding assay used to index GABA neuronal uptake sites, shows no evidence of widespread reductions, even in autopsy samples (Spillane *et al.*, 1977; Smith *et al.*, 1983; Simpson *et al.*, 1988). We have previously measured the release of SLIR in brain biopsy tissue and no reduction was found (Francis *et al.*, 1987*a*). The cholinergic deficiency hypothesis is also supported by evidence that increased activity of remaining monoaminergic neurons occurs (reviewed by Palmer *et al.*, 1987). The changes of cholinergic under-activity are most closely related to the clinical course of AD (Francis *et al.*, 1987*a*, *b*; Palmer *et al.*, 1987; Sims and Bowen, 1987). Thus the deficits reported postmortem for GABA and other neuroactive substances may not require therapeutic correction until the later stages of the disease to provide significant clinical improvement.

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ACTIVATION OF NECK MUSCLES FROM THE HUMAN MOTOR CORTEX

by S. C. GANDEVIA *and* C. APPLEGATE[1]

(*From the Unit of Clinical Neurophysiology, Department of Neurology, The Prince Henry Hospital and School of Medicine, University of New South Wales, Sydney, Australia*)

SUMMARY

Percutaneous stimulation of the motor cortex has been used to assess directly the supranuclear projection to the sternomastoid, trapezius and splenius capitis muscles. The projection to sternomastoid had a mean latency of 6.5 ms for the contralateral electromyographic response. A smaller and more variable response, usually with a longer latency (mean 9.5 ms), occurred in the ipsilateral sternomastoid. Electromyographic responses on both sides were potentiated by voluntary contraction or strong inspiratory efforts. They were evoked at lower stimulus intensities in the contralateral sternomastoid. Short-latency responses were recorded from the contralateral but not the ipsilateral trapezius and splenius capitis muscles. These results indicate that weakness of head rotation towards the hemiplegic limb following a supranuclear lesion may reflect reduced power of dorsal neck muscles rather than of sternomastoid.

INTRODUCTION

Percutaneous stimulation of the motor cortex or immediately subjacent corticofugal axons with high-amplitude electrical or magnetic pulses permits investigation of short-latency pathways to descending motoneuron pools (Merton and Morton, 1980; Barker *et al.*, 1985). A number of studies have described the properties of these presumed corticospinal pathways to distal muscles in the upper and lower limb (e.g., Marsden *et al.*, 1983; Rossini *et al.*, 1985*a*, *b*; Hess *et al.*, 1987; Rothwell *et al.*, 1987). However, a recent study has documented a powerful oligosynaptic pathway to the phrenic and intercostal motoneuron pools (Gandevia and Rothwell, 1987). The latter study has emphasized that the human motor cortex can access not only motoneurons of distal muscles at short latency, but also those of respiratory and proximal limb muscles.

Although the outflow to cranial nerve nuclei is technically difficult to study with percutaneous cortical stimulation, cortical outflow to the sternomastoid muscle is of particular interest. First, it is widely held that the motor cortex controls

Correspondence to: Dr S C Gandevia, Unit of Clinical Neurophysiology, The Prince Henry Hospital, PO Box 233, Matraville, Sydney, Australia 2036

[1] Present address: Department of Neurology and Neurological Surgery, Washington University School of Medicine, St Louis, Missouri, USA.

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contralateral head turning via an effectively ipsilateral pathway to sternomastoid (e.g., Balagura and Katz, 1980; Geschwind, 1981; Willoughby and Anderson, 1984) although the neuroanatomical substrate for this remains unclear—both a direct ipsilateral pathway (Balagura and Katz, 1980; Mastaglia *et al.*, 1986) and a pathway with a double decussation (Geschwind, 1981) have been suggested. The classical evidence for an ipsilateral pathway is that patients with a destructive lesion of one cerebral hemisphere have weakness of rotation of the head towards the hemiplegic (or hemiparetic) side (e.g., Beevor, 1909; Balagura and Katz, 1980; Willoughby and Anderson, 1984; Mastaglia *et al.*, 1986). Studies of head movement induced by direct cortical stimulation (e.g., Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950; Penfield, 1958; Bender *et al.*, 1964; for review *see* Tasker *et al.*, 1982) and by epileptic seizures (e.g., Robillard *et al.*, 1983; Ochs *et al.*, 1984) have not fully supported the concept of ipsilateral control of the sternomastoid muscle. Some neurological and neuroanatomical texts favour a bilateral or effectively ipsilateral projection (e.g., Patten, 1977; Snell, 1980; Spillane and Spillane, 1982; Haymaker and Kuhlenbeck, 1976) but others suggest that a contralateral projection may be important (e.g., DeJong, 1967; Barr, 1972; Brodal, 1981). A second interesting feature of this muscle is that it is involved in two types of head movement (rotation to one side and lateral flexion to the other, Williams and Warwick, 1980) in addition to its role as an inspiratory muscle which elevates the thorax when respiratory drive is increased (Tokizane *et al.*, 1951; Campbell, 1955, 1958). One case report implies that different neural pathways to the sternomastoid may be activated during voluntary head rotation and neck flexion (Iannone and Gerber, 1982). The present study provides the first electrophysiological evidence for a powerful contralateral projection to specific neck muscles from the motor cortex. A weaker ipsilateral projection was also found.

METHODS

Twelve successful studies were conducted on 5 subjects, 2 of whom were studied on a number of occasions. The experimental procedures were approved by local ethics committees. All studies were conducted in an air-conditioned and electrically isolated laboratory. Subjects were studied while seated in a comfortable chair or in the supine position. When required, padding and pillows were used to support the head and neck in a comfortable 'rest' position.

Stimulation and recording techniques

Indirect activation of corticofugal pathways was achieved using percutaneous stimulation (Merton and Morton, 1980). Electrodes were routinely fixed to the scalp with collodion at the following locations: vertex, 6 cm anterior to the vertex, 6 cm lateral to the vertex and 3–4 cm anterior to the 'lateral' electrode. The stimulator (Devices D180) delivered single stimuli up to 750 V (time constant: 50 μs or 100 μs). In the initial studies the cathode was at the vertex and the anode at sites 3–6 cm lateral to it. As the largest responses in contralateral neck muscles were achieved with the anode located laterally, in subsequent studies stimulation was delivered with the anode 6 cm lateral to the vertex and the cathode 3–6 cm anterior to it ('lateral' bipolar stimulation). With interelectrode distances of 3–4 cm higher stimulus intensities (up to 600 V) were required to activate contralateral hand and neck muscles. This technique was effective in restricting the stimulus current to the appropriate hemisphere.

Recordings of electromyographic (EMG) activity were made with bipolar wire electrodes. Two insulated stainless steel wires (50 μm diameter) with 1 mm of insulation removed were inserted into the muscle to an approximate depth of 15 mm. Electrodes were placed in both sternomastoid muscles at the junction of the lower third and upper two-thirds (7 studies), both trapezius muscles in the upper portion at a level opposite the spinous process of the fourth cervical vertebra (3 studies), and both splenius capitis muscles at the level of the third cervical spinous process (2 studies). In all subjects the medial and lateral edges of the sternomastoid were carefully marked at the level at which electrodes were inserted. The location of the electrodes was checked using movements designed to activate selectively the muscles under study. These included shoulder elevation, forward and lateral flexion of the neck, extension of the neck, head rotation and arm abduction. The recording depth and activation by specific movements assured that electrodes within the sternomastoid were deep to platysma. The electrodes recorded multiunit activity involving at least 4 motor units during weak contractions with a fuller interference pattern during stronger contractions. In one study a standard concentric needle electrode was used and in another surface electrodes were positioned over sternomastoid and trapezius. Concentric electrodes could not easily be held in the same intramuscular site for sufficient periods of time to complete the study and surface electrodes were not used routinely because of the stimulus artefact produced by the high-voltage stimuli. In 4 studies EMG was also recorded from ipsilateral and contralateral thenar muscles or from contralateral tibialis anterior using surface electrodes.

EMG activity was amplified and filtered (bandpass 80 Hz–1.6 kHz). Usually the EMG was full-wave rectified before it was averaged (sampling rates 3–30 kHz) for 12.5–25 ms prior to the stimulus until 37.5–75 ms after it (*see* fig. 1) All sweeps were viewed on a digital oscilloscope with a pretrigger facility and those which were contaminated by large amplitude motor unit potentials in the vicinity of the trigger were usually rejected. The stimulus artefact varied between experimental sessions and within a session if several sets of bipolar wires were used. In one study the initial artefact was not detectable (fig. 1) but in all others it was noticeable (figs 2–6). No attempt was made to remove the stimulus artefact electronically but particular attention was paid to connection of the fine wire electrodes to the skin-mounted preamplifier and to the position of a lead 'collar' through which the subject was earthed. The collar was usually just above the exit of the electrodes from the skin. Raw data were recorded on tape for reanalysis. Sets of 4–10 consecutive responses were averaged and a number of sets of responses were averaged from each study. The latency was determined from the first consistent rise in the averaged rectified recordings measured using a cursor and this corresponded closely with that measured from optimal single trials. For figures in which only averaged rectified EMG is shown (figs 2–4, 6) the background level did not exceed 20% of the amplitude of the evoked responses.

RESULTS

Wire electrodes were inserted into the sternomastoid, splenius or trapezius muscles on both sides (*see* Methods). In each subject single stimuli delivered to the motor cortex through electrodes lateral to the vertex activated neck muscles on the contralateral side. Details of the projection revealed by percutaneous stimulation are given below.

The best responses were obtained in the neck muscles contralateral to the site of stimulation when the anode was located 6 cm lateral to the vertex with the cathode anterior to the lateral electrode or at the vertex. The lateral location of the anode was also optimal for stimulation of contralateral muscles in the forearm and hand but was lateral to the optimal location for muscles acting at the shoulder. With the cathode at the vertex there was activation of contralateral hand and neck muscles at stimulus intensities of 300–400 V (50 μs decay, *see* Methods) with no overt action on proximal muscles of the upper limb. Anodal stimulation at the vertex produces optimal activation of lower limb muscles (e.g., Rossini *et al.*, 1985*b*; Rothwell *et al.*, 1987).

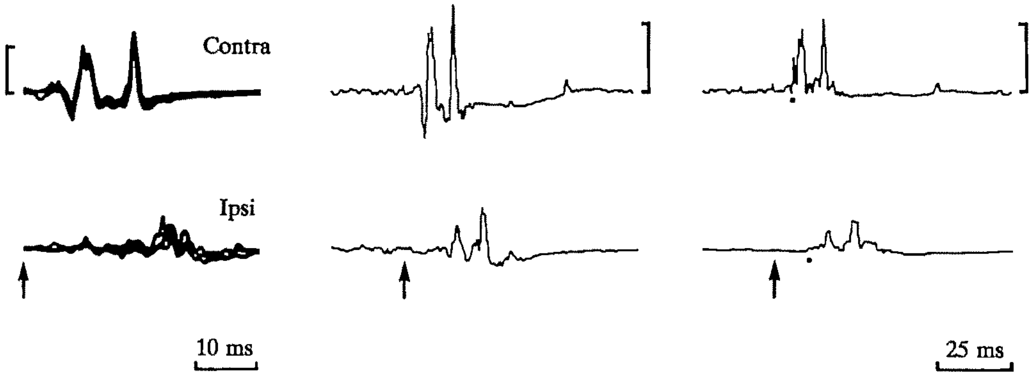

FIG. 1. Responses recorded simultaneously from both sternomastoid muscles with intramuscular electrodes in one subject. *Left panels* show 4 consecutive responses superimposed *Middle panels* show the average of the 4 responses. *Right panels* show the average of the rectified EMG with points at which latencies were measured marked by a dot. Stimuli were delivered at times indicated by the arrow through an anode located 6 cm lateral to the vertex with the cathode 4 cm anterior to the anode (*see* Methods) during simultaneous isometric contraction of both contralateral and ipsilateral sternomastoid muscles Stimulus intensity: 500 V, 50 μs decay. Vertical calibration· 100 μV Responses were evoked bilaterally but were larger, and at shorter latency, on the contralateral side.

Fig. 1 shows typical results from one set of 4 stimuli in one subject. Stimuli were delivered during weak forward flexion of the neck which activated motor units in both muscles. They evoked a reproducible response in the contralateral sternomastoid at a latency of 6.5 ms in addition to a smaller more variable response in the ipsilateral sternomastoid at a latency of about 13 ms. Because of the variable activation of the ipsilateral muscle, latencies were usually measured from averages of rectified EMG (fig. 1, at right). Data for the projection to sternomastoid from other subjects are shown in figs 2–4. The contralateral responses following cortical stimulation were often polyphasic, particularly at high stimulus intensities. The duration of the cortically evoked responses was often longer than that expected for compound action potentials produced by nerve stimulation and recorded with concentric or bipolar wire electrodes (e.g., Fahrer *et al.*, 1974; Pertrera and Trojaborg, 1984; Gandevia and Plassman, 1988). This is shown for one subject in fig. 4 for sternomastoid. Responses from this subject consistently showed 3–4 segmented peaks in the EMG.

The minimal latency observed in each subject for a set of stimuli ranged from 4.5 ms to 9.8 ms with a mean of 6.5 ms (±0.8 ms, SEM) in the contralateral sternomastoid and from 4.5 ms to 14.5 ms with a mean of 9.5 ms (±1.7 ms) in the ipsilateral sternomastoid. In one subject no definite ipsilateral response was evoked within the first 30 ms following the stimuli although an EMG burst usually occurred at a latency of about 40 ms. The latency of the minimal ipsilateral response recorded in one session for 3 subjects *exceeded* that of the contralateral response by 2.0, 6.8 and 8.0 ms. For 6 sets of stimuli in one subject the responses occurred in both muscles at short latency in 3 sets (*see* fig. 3) and in the other 3 the latency remained at 5.0–6.4 ms on the ipsilateral side but was longer on the contralateral side (7.3–11.5 ms). The observed variation in latency between successive trials would be greater with the intramuscular electrodes used here than with conventional surface electrodes. For technical reasons the latter electrodes could not be used routinely in this study. Variation between subjects in the minimal latencies may reflect in part slight differences in the background level of contraction.

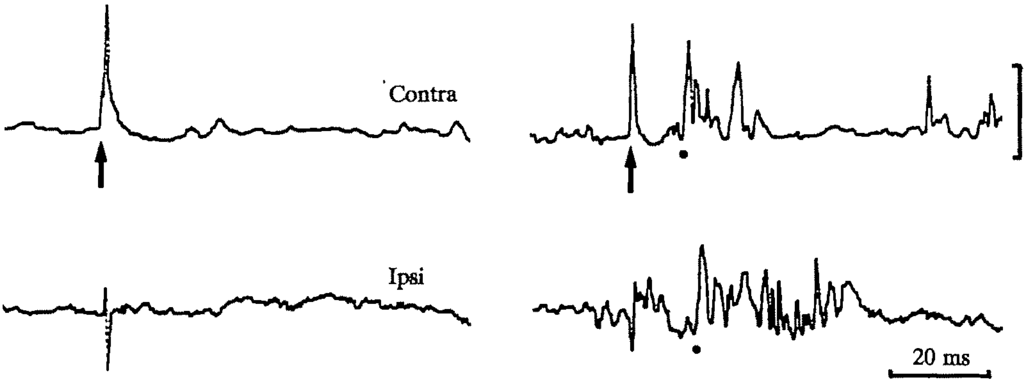

FIG 2. Responses recorded simultaneously from the contralateral (*upper traces*) and ipsilateral sternomastoid (*lower traces*) to show the potentiation produced by a background voluntary contraction Averages of rectified EMG based on 4 trials (375 V, 50 μs decay) when relaxed (*left panels*) and during a bilateral contraction (*right panels*). Stimuli delivered at the arrow. Vertical calibration: 200 μV.

As described previously for limb and truncal muscles (e.g., Merton and Morton, 1980; Rossini *et al.*, 1985*a*; Gandevia and Rothwell, 1987; Rothwell *et al.*, 1987), the responses evoked by the cortical stimuli were potentiated by voluntary activation of the motoneuron pool. Fig. 2 shows an example of the potentiation of the responses to a background voluntary contraction which activated the sternomastoid on both sides. Identical stimuli (375 V, 50 μs decay) were delivered at rest (left panel) and during a bilateral contraction (right panel). Little or no definite response was evoked when the muscle was completely relaxed during voluntary apnoea but clear responses were produced bilaterally when stimuli were delivered during a voluntary contraction. This establishes that the responses were neurally mediated and were not evoked by direct activation of nerves at extracranial sites. Fig. 4 depicts data which confirm this finding for the contralateral projection in another subject. The ipsilateral EMG response is unlikely to represent 'field spread' from contralateral muscles given the use of bipolar wire electrodes and the usual difference in latency between the responses. Whether the contralateral sternomastoid was active during forward flexion of the neck, lateral flexion to the contralateral side, rotation of the head to the ipsilateral side or sustained inspiratory efforts, the cortical stimulus evoked a short-latency EMG response.

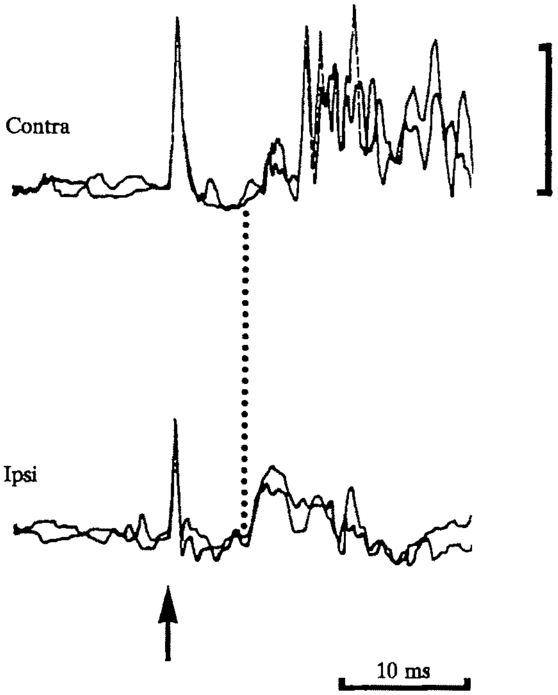

FIG 3. Responses in both sternomastoid muscles following stimulation of the scalp through a pair of lateral electrodes. Stimuli were delivered (at the arrow) during weak isometric neck flexion. Each trace (rectified EMG) is an average of 4 responses and 2 traces are superimposed There was a bilateral response with a similar latency in both muscles. A dotted line is drawn through the approximate onset of the responses. Stimulus intensity· 700 V, 50 μs decay. Vertical calibration: 100 μV.

At the start of each study the stimulus intensity was increased until a detectable response occurred in either muscle. In all subjects it was not easy to achieve complete electromyographic silence in sternomastoid whether in the lying or sitting position, with or without full support of the head and neck. Thus it is difficult to provide the *absolute* threshold intensity required to activate the relaxed sternomastoid. However, during weak voluntary contractions the contralateral response was usually evident at a lower stimulus intensity (by 100–300 V) than that required for the ipsilateral sternomastoid. This observation and the modal latencies of the responses suggest that the stimuli probably had a more powerful effect upon contralateral than ipsilateral sternomastoid motoneurons. It is possible

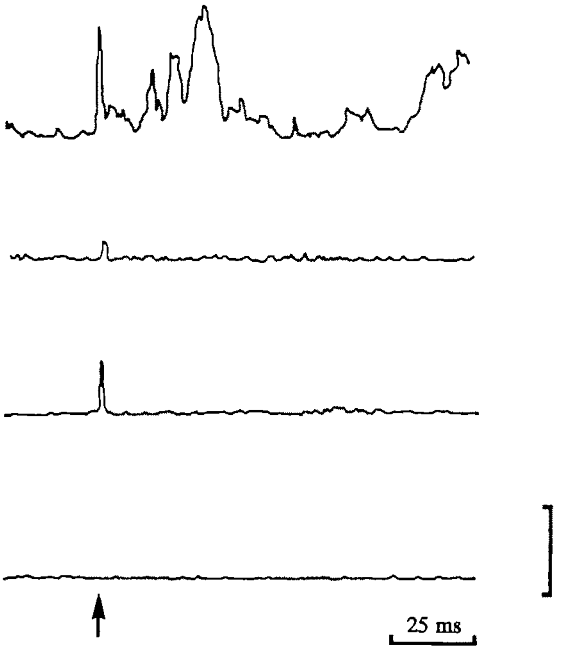

FIG. 4. Responses recorded from the contralateral sternomastoid (*top 3 traces*) and tibialis anterior (*bottom trace*) to cortical stimuli delivered through lateral scalp electrodes (*see* Methods). The responses in sternomastoid were recorded during a strong voluntary inspiratory effort against occlusion at functional residual capacity (*upper trace*), during the inspiratory phase of tidal breathing (*second trace*) and during voluntary apnoea with no background activity in sternomastoid (*third trace*). The same intensity stimuli produced no response in the contralateral tibialis anterior (during voluntary contraction). All traces are averages of 4 rectified responses. Vertical calibration. 100 μV.

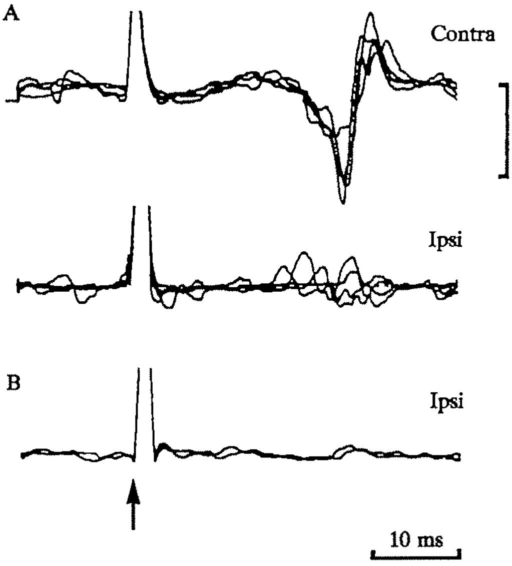

FIG. 5. Responses recorded simultaneously from upper trapezius on both sides with intramuscular electrodes in one subject. Timing of stimuli indicated by the arrow A, 4 superimposed responses of unrectified EMG. B, duplicate averages of 8 rectified EMG responses of the ipsilateral muscle when it was strongly activated in lateral flexion or rotation of the head against resistance. A consistent short-latency response is not evident for the ipsilateral trapezius. Vertical calibration: 100 μV

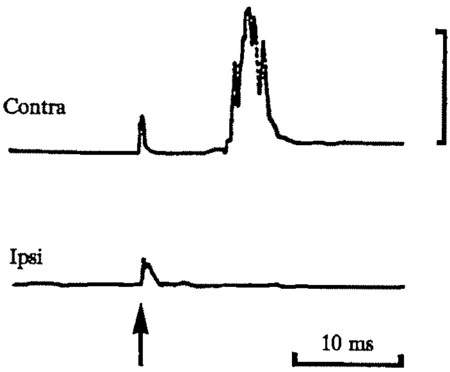

FIG. 6. Responses recorded simultaneously from splenius capitis with intramuscular electrodes in one subject. Each trace is the average of 8 rectified EMG responses. Stimuli were delivered (at the arrow) during voluntary contraction which activated both muscles. No short-latency ipsilateral responses were evoked by the stimuli. Vertical calibration. 150 μV.

that the similarity in stimulus level for threshold activation of neck and thenar muscles reflects difficulty in achieving full relaxation in the neck muscles.

A projection to the splenius capitis and upper trapezius muscles was sought using similar techniques in repeated studies on 2 subjects. A definite contralateral projection was documented for trapezius with latencies of 5.2 to 7.7 ms in 1 subject (mean 6.3 ms) and 7.6 to 10.0 ms (mean 8.7 ms) in the other (fig. 5A). In only 1 of the 16 sets of stimuli was there evidence for an ipsilateral response in trapezius with a latency less than 30 ms. For studies in which the responses in both sternomastoid and trapezius were measured, the mean latencies were shorter in the contralateral sternomastoid by about 2 ms (range 0.1–4.5 ms). Similarly a corticofugal projection to the contralateral splenius capitis was documented (latencies of 11.2 and 12.5 ms) but not on the ipsilateral side (fig. 6). A reproducible short-latency response in the ipsilateral trapezius and splenius capitis was not unmasked by strong voluntary contraction of the muscles (fig. 5B) and use of the maximal output from the stimulator (750 V, 100 μs decay, *see* Methods).

DISCUSSION

The motor cortex appears to have a strong short-latency projection to motoneurons supplying the contralateral sternomastoid, trapezius and splenius capitis muscles. Two properties of this projection are shared by that to proximal and distal muscles of the upper limb, namely its comparatively short latency and its potentiation by voluntary activation of the motoneuron pool (e.g., Marsden *et al.*, 1983; Rossini *et al.*, 1985*a*; Gandevia and Rothwell, 1987; Rothwell *et al.*, 1987). The corticofugal projection can access the sternomastoid motoneuron pool both during voluntary head movements which activate the muscle and during inspiratory manœuvres.

Evidence for a relatively weak 'projection' to the ipsilateral sternomastoid was obtained and the mechanisms underlying this projection are now discussed. It is important to consider whether the ipsilateral responses represented activation via stimulus spread to intramuscular nerve fibres or even direct muscle activation. Such activation may occur for muscles close to, or under, the electrodes (for example, temporalis and frontalis), but it is unlikely to be responsible for all ipsilateral responses observed in the present study (*see also* Merton *et al.*, 1981). First, the responses were usually evoked at longer latencies than for the contralateral sternomastoid (fig. 1). They were not noted for trapezius and splenius capitis. Secondly, ipsilateral (and contralateral) responses were facilitated by a background contraction involving the pools of sternomastoid motoneurons (fig. 2). Thirdly, the responses were usually larger in the contralateral muscle. Furthermore, effective activation of sternomastoid bilaterally occurred with little or no stimulus spread to the 'leg area' near the vertex and without activation of ipsilateral hand muscles. Finally, while the exact site at which electrical stimuli activate corticofugal pathways is unknown (*see* Day *et al.*, 1987), the observed responses follow loosely the established somatotopic organization for the primate motor cortex (e.g., Leyton and Sherrington, 1917; Foerster, 1936; Penfield and Boldrey, 1937; Phillips and Porter, 1977) so that activation of deep midline structures is unlikely to be responsible for the bilateral activation of sternomastoid described above.

The technique of percutaneous cortical stimulation cannot define the specific neural pathways involved in the activation of the neck muscles. However, data from previous studies of the human upper limb suggest that each stimulus evokes one (or more) rapid corticofugal volleys (Boyd *et al.*, 1986; *see also* Calancie *et al.*, 1987; Rothwell *et al.*, 1987). The suggestion is consistent with results obtained in subhuman primates with direct and indirect stimulation of the cortex (Landgren *et al.*, 1962; Phillips and Porter, 1977; Ludolph *et al.*, 1987). Segmented EMG responses of relatively long duration were noted in the present study. The results obtained here argue for a rapid oligosynaptic projection to the sternomastoid motor nucleus. The requirements for at least 2 synaptic delays, probable ascent via an accessory spinal pathway (*see* Hayward, 1986) and the estimates of central conduction time from the cortex to the midcervical (Gandevia and Rothwell, 1987) and lower cervical cord (Mills and Murray, 1985; Rothwell *et al.*, 1987; Gandevia and Plassman, 1988) of about 5 ms emphasize that the minimal latencies observed for sternomastoid (about 6.5 ms) are appropriately short. Latencies of 5 to 7 ms for evoked responses in the contralateral sternomastoid have recently been recorded in another laboratory (J. C. Rothwell, personal communication, 1987). These latencies may be compatible with a monosynaptic contribution to the evoked responses presumably via corticospinal fibres or possibly corticobulbar fibres (Hayward, 1986). Ascent of the phylogenetic scale may be associated with increased innervation of trapezius and sternomastoid by the more direct pathway via cervical nerves (Straus and Howell, 1936; *see also* Fahrer *et al.*, 1974) and there is some evidence for a motor cortical projection to the region of the spinal accessory nucleus in man (Kuypers, 1958). Stimulus artefact prevented the use of spinal stimulation to estimate the peripheral components of the latency of responses in neck muscles to cortical stimulation. However, given that the upper limit of the latency in upper trapezius to stimulation of the accessory nerve is 3 to 3.5 ms (Fahrer *et al.*, 1974; Pertrera and Trojaborg, 1984) and that cortically-evoked responses occurred at 6.3 to 8.7 ms, the 'central' conduction time (including synapse(s) and conduction to the posterior triangle of the neck) is about 3 to 4 ms. Estimates of central conduction time for anterior deltoid and the diaphragm are about 4 ms (Gandevia and Rothwell, 1987), 4.5 ms for biceps brachii and about 5 ms for the intrinsic muscles of the hand obtained using similar techniques (Rothwell *et al.*, 1987; Gandevia and Plassman, 1988).

Given the pattern of innervation revealed in the present study, it is possible to explain the clinical observation of a slight 'weakness' of head rotation towards the hemiplegic side in patients with cortical or internal capsule lesions. This weakness may reflect loss of a projection to contralateral posterior neck muscles (including splenius) rather than loss of a dominant projection to the ipsilateral sternomastoid. In addition to the direct evidence from the present study, evidence *against* a dominant ipsilateral projection to sternomastoid comes from some studies of patients during seizures and direct cortical stimulation (*see* Introduction). Such a suggestion requires that the large posterior neck muscles act as powerful rotators of the head. The size and wide insertion of splenius capitis make it suited to this task in human subjects (Williams and Warwick, 1980) and in the cat (Richmond and Abrahams, 1975; Richmond *et al.*, 1985). Certainly these posterolateral muscles are activated during head rotation and lateral flexion to the same side. If the contralateral projection to these muscles was the major source of voluntary drive, patients with hemiplegia might be expected to have virtual paralysis of the contralateral trapezius: this has been observed (Wernicke, 1889). Although the electrophysiological study suggests that the projection to the ipsilateral sternomastoid is synaptically less 'efficient' than that to the contralateral side, it does not necessarily imply that the contribution to voluntary strength on the ipsilateral side is proportionately weak.

Finally, the electromyographic studies indicated that the individual neck muscles are activated voluntarily in complex ways according to the exact position of the head and the desired movement. For example, many subjects initially contracted both sternomastoid muscles on attempted head rotation due to the unintentional lateral flexion of the head, and commonly when subjects were sitting there was background activity in both muscles dependent upon head position. Clinical assessment of the strength of neck movements will be complicated by such effects. Indeed, the apparent 'strength' of neck rotation can be adjusted by different degrees of lateral neck flexion. Furthermore, unless full activation of motor units in neck muscles can normally be achieved in maximal voluntary efforts (as occurs for limb and respiratory muscles; *see* Merton, 1954; Belanger and McComas, 1981; Gandevia and McKenzie, 1985), difficulties in estimates of strength of neck muscles are increased.

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Note added in proof: Recent evidence obtained using transcranial magnetic stimulation of the motor cortex supports the presence of a contralateral projection to sternomastoid with a relatively weaker ipsilateral projection (R. Benecke, B.-U. Meyer, P. Schönle and B. Conrad, *Experimental Brain Research*. In press.

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LIPID STORAGE MYOPATHY ASSOCIATED WITH LOW ACYL-CoA DEHYDROGENASE ACTIVITIES

by D. M. TURNBULL,[1] I. M. SHEPHERD,[1,2] B. ASHWORTH,[4]
K. BARTLETT,[3] M. A. JOHNSON,[1] M. J. CULLEN,[1] S. JACKSON[1,3]
and H. S. A. SHERRATT[2]

(*From the* [1]*Muscular Dystrophy Laboratories, Department of Neurology,* [2]*Department of Pharmacological Sciences and* [3]*Department of Child Health, University of Newcastle upon Tyne, and* [4]*Department of Clinical Neurosciences, University of Edinburgh*)

SUMMARY

A man with a painful proximal myopathy had excess lipid deposition in skeletal muscle, excretion of dicarboxylic acids in urine and low acyl-CoA dehydrogenase activities in skeletal muscle mitochondria. In addition he had little immunoreactive short-chain and medium-chain acyl-CoA dehydrogenase enzyme protein compared with normal controls. Following treatment with riboflavin there was considerable improvement in his clinical condition which was confirmed by further biochemical and morphological investigations.

INTRODUCTION

There has been a considerable increase in knowledge over the last few years about a group of muscle diseases associated with defects in fatty acid oxidation (Turnbull *et al.*, 1987). Until recently, patients were classified as having a lipid storage myopathy with or without carnitine deficiency (DiMauro *et al.*, 1980). Techniques are now available for the comprehensive investigation of these patients, including detailed analysis of intermediary metabolites and carnitine esters in plasma and urine, and the measurement of the individual enzymes of β-oxidation. An increasing number of defects of the various acyl-CoA dehydrogenases, the enzymes which catalyse the first step of β-oxidation, are being recognized (Stanley *et al.*, 1983; Turnbull *et al.*, 1984; Hale *et al.*, 1985; Zierz *et al.*, 1986). It is likely that many patients previously reported with lipid storage myopathy have defects involving these enzymes.

CASE HISTORY

R.K. presented in 1985, at the age of 39 yrs, with a 2 yr history of muscle pain and weakness. This started with pain in the extensor muscles of his neck and then spread to involve deltoid and

Correspondence to: Dr D. M. Turnbull, Muscular Dystrophy Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

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the other muscles of the shoulder girdle. He developed weakness of his upper limbs which was more prominent at the end of the day or following strenuous exercise. This weakness progressed and he could not continue to work as a toolmaker. Over the few months before admission to hospital he noticed pain in his legs on walking and difficulty rising from low chairs. He had no muscle cramps or contractures on exercise nor any episodes of myoglobinuria.

His past medical history was unremarkable. His motor milestones were normal and he had actively participated in sport without problems. He had continued to play football until the age of 32 yrs, and ceased playing golf at 37 because of his present neuromuscular problem. There was no family history of metabolic or neuromuscular disease.

There was weakness of neck muscles and the proximal limb muscles. Mild wasting of the proximal upper limb muscles and generalized muscle tenderness were present. The tendon reflexes were intact.

The results of routine haematological and biochemical investigations were normal. His serum creatine kinase (CK) activity was elevated at 666 U/l (normal range < 175). Electrophysiological studies showed normal nerve conduction velocities. Electromyography (EMG), including single fibre and Macro EMG, indicated an active myopathy (Dr P. R. W. Fawcett). An open muscle biopsy was performed and he was started on riboflavin 25 mg thrice daily increasing after 3 months to 50 mg thrice daily.

R.K. was readmitted for further investigation in July 1986. He had continued on riboflavin therapy. His symptoms had decreased although he still suffered muscle pain on exertion. His muscle strength had improved. The results of the routine investigations were normal but the serum CK was slightly elevated at 181 U/l. Neurophysiological studies showed an improvement with only minimal changes present on this occasion.

METHODS

Metabolic response to fasting

The subject and 5 controls fasted for 36 h and blood samples were taken through an indwelling cannula. The blood metabolite concentrations were determined fluorometrically (Lloyd *et al.*, 1978).

Histochemistry and electron microscopy

Muscle biopsies were obtained from the patient before and after 15 months of treatment. Some of the tissue was frozen in dichlorodifluoromethane (Arcton 12, ICI) cooled to −150° C in liquid nitrogen. Serial transverse sections, 10 μm thick, were cut on a cryostat microtome and used to demonstrate the activity of myofibrillar ATPase (mATPase) after preincubation at pH 4.3, 4.6 or 10.2 (Brooke and Kaiser, 1970). Sudan Black B and Oil Red O methods (Lillie, 1965) were used to screen for the presence and distribution of excess tissue lipid. Other histochemical procedures were as described previously (Johnson, 1983). Preparation of tissue for electron microscopy was by standard techniques used in our laboratories (Cullen and Fulthorpe, 1982).

Organic acids

Organic acids in urine were analysed as their methyl ester by gas liquid chromatography mass spectrometry.

Mitochondrial oxidations and cytochrome concentrations

Mitochondrial fractions were prepared from fresh skeletal muscle in 1985 as described by Johnson *et al.* (1983), and in 1986 as described by Sherratt *et al.* (1988). Substrate oxidations were recorded spectrophotometrically at 420–475 nm at 30° C with 10 mM succinate or with 10 mM glutamate plus 1 mM malate as substrate, using 0.5 mM ferricyanide as final electron acceptor (Turnbull *et al.*, 1982). Rotenone was included when succinate was the substrate. Cytochrome concentrations were determined from the reduced-oxidized difference spectra of skeletal muscle mitochondrial fractions at −190° C with succinate as reductant (Sherratt *et al.*, 1988). Protein was determined by a modified Lowry method (Sherratt *et al.*, 1988).

Measurement of acyl-CoA dehydrogenase activities

Acyl-CoA dehydrogenase activities were measured in human skeletal muscle mitochondria by 2 different methods. The acyl-CoA dehydrogenase activity of the mitochondrial fraction prepared from the first biopsy was assayed spectrophotometrically using a dye reduction method with phenazine methosulphate as an intermediate electron acceptor and dichlorophenolindophenol as final acceptor (Turnbull *et al.*, 1984). This method has a high background rate and requires a relatively large amount of mitochondrial protein. Frerman and Goodman (1985*a*) devised an ETF-linked fluorometric assay which is both more specific and more sensitive. This assay was used to determine the acyl-CoA dehydrogenase activity in both the first and second mitochondrial fractions. Skeletal muscle mitochondria (approximately 750 μg protein) were incubated for 20 min on ice with 10 volumes of 25 mM potassium phosphate, 0.2 mM EDTA, 0.5% (w/v) Triton X-100; pH 7.4 and centrifuged at 11 000 g for 10 min. The supernatant containing the acyl-CoA dehydrogenases was washed twice with peroxide-free ether to remove dispersed lipids. The washed supernatant was diluted appropriately before assay with 25 mM potassium phosphate, 0.2 mM EDTA; pH 7.4.

Immunoblot analysis of acyl-CoA dehydrogenases

Short-chain and medium-chain acyl-CoA dehydrogenases were purified from 2 kg of pig liver using the method described by Ikeda *et al.* (1985). These enzymes were used to raise antibodies in New Zealand White rabbits. The anti-(short-chain acyl-CoA dehydrogenase) antibodies and anti-(medium-chain acyl-CoA dehydrogenase) antibodies specifically precipitated the enzymes dehydrogenating butyryl-CoA and octanoyl-CoA respectively. These antibodies cross-reacted with the appropriate enzymes in mitochondrial extracts prepared from human liver, kidney, heart and skeletal muscle (I. M. Shepherd and D. M. Turnbull, unpublished observations).

SDS-polyacrylamide gel electrophoresis was performed on 10% separating gels (Laemmli, 1970). The proteins were transferred electrophoretically from the gel to a nitrocellulose filter as described by Towbin *et al.* (1979) with detection using the peroxidase-antiperoxidase method with 4-chloro-1-naphthol as substrate (Domin *et al.*, 1984).

RESULTS

Blood metabolites

On fasting, the concentration of 3-hydroxybutyrate increased to more than 3 times the control mean (Table 1). The lactate and pyruvate concentrations were also elevated but were less marked than the ketone body concentration. The concentrations of 3-hydroxybutyrate and acetoacetate were normal after the

TABLE 1 BLOOD METABOLITE CONCENTRATIONS AFTER 36 HOURS FASTING

	Glucose (mM)	*Lactate (mM)*	*Pyruvate (mM)*	*Alanine (mM)*	*Glycerol (mM)*	*3-Hydroxy-butyrate (mM)*	*Acetoacetate (mM)*
Patient							
1985	4.29	1.06	0.084	0.166	0.044	2.46	—
1986	3.99	0.938	0.072	0.207	0.072	0.710	0.290
Controls							
Mean	3.91	0.62	0.059	0.185	0.088	0.748	0.260
SD	±0.67	±0.08	±0.013	±0.069	±0.037	±0.365	±0.107

fast in 1986. There were small increases in lactate and pyruvate concentrations (Table 1).

Urinary metabolites

The fasting urine examined in 1985 contained abnormal amounts of ethylmalonic, methylsuccinic and adipic acids (31, 5, 2 μmol/mmol creatine, respectively) (fig. 1). These compounds are normally undetectable in urine from adult subjects. No other abnormal compounds were detected by gas chromatography. The urine examined in 1986 contained no detectable abnormal metabolites.

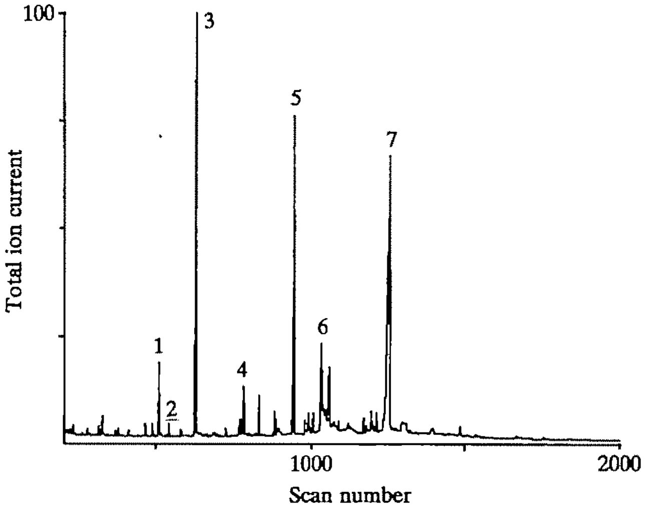

FIG. 1. Gas chromatogram of urinary organic acids. 1, ethylmalonate; 2, methylsuccinate; 3, octanoate (internal standard); 4, adipate; 5, phenylbutyrate (internal standard); 6, citrate; 7, hippurate.

Histochemistry

The initial pretreatment biopsy showed an abnormal degree of variability of fibre size due to disseminated fibre atrophy mainly affecting the type 1 fibres. Many type 1 fibres also showed vacuolation due to increased storage of neutral lipid demonstrated with Oil Red O (fig. 2A). Subsarcolemmal proliferation and clustering of mitochondria was seen in sections demonstrating succinate dehydrogenase activity (fig. 2B). Aggregations of mitochondria were also visible in Sudan Black B preparations but Oil Red O staining showed that the areas of mitochondrial aggregation were not selectively affected by neutral lipid deposition. The lipid storage and mitochondrial abnormalities were sufficiently severe to give vacuoles and clefts in muscle sections stained using conventional histological techniques (e.g., haematoxylin and eosin, *see* fig. 2C).

After treatment the muscle became both morphologically and histochemically more normal. The content of neutral lipid was greatly decreased but there were still some minimal irregularities of mitochondrial distribution. In conventional histological preparations vacuolation and cleft formation was no longer apparent.

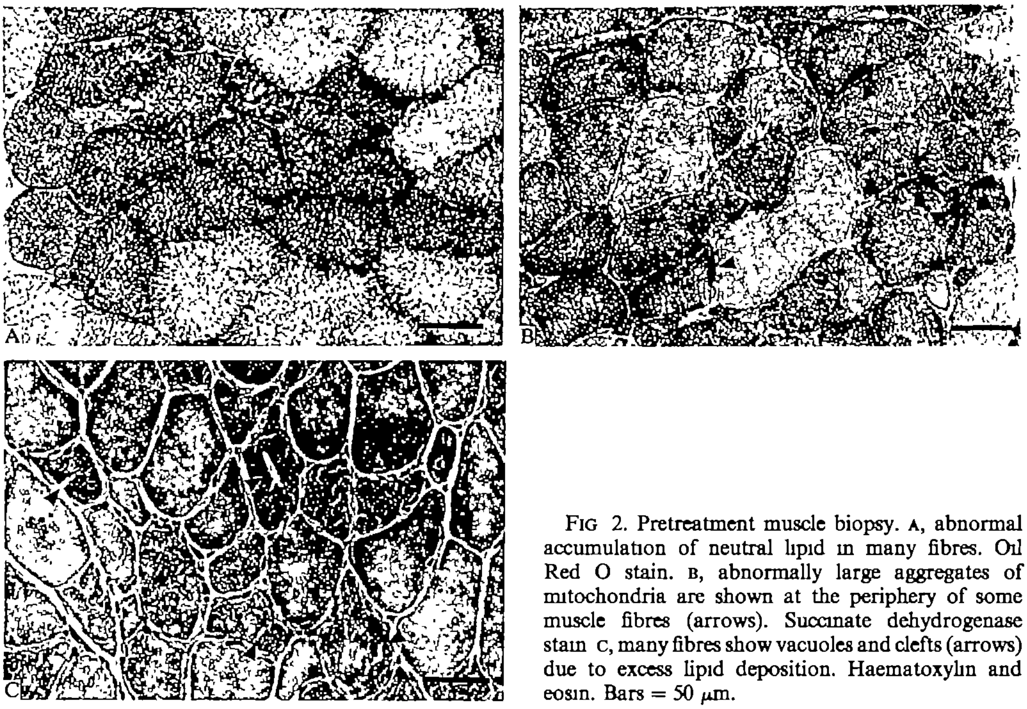

FIG 2. Pretreatment muscle biopsy. A, abnormal accumulation of neutral lipid in many fibres. Oil Red O stain. B, abnormally large aggregates of mitochondria are shown at the periphery of some muscle fibres (arrows). Succinate dehydrogenase stain C, many fibres show vacuoles and clefts (arrows) due to excess lipid deposition. Haematoxylin and eosin. Bars = 50 μm.

However, the percentage of muscle fibres with internal muscle nuclei was very high (approx. 30–40%); this abnormality was not present in the original biopsy but did not seem to be related to any ongoing or previous muscle fibre necrosis since there was no histological evidence of frank muscle fibre necrosis in either biopsy.

Electron microscopy

Lipid droplets located between the myofibrils were frequently seen in the pretreatment biopsy, in some fibres forming up to 10% of the volume. In these fibres the droplets often also formed subsarcolemmal aggregates resulting in some disruption of the myofibrillar architecture (fig. 3). The droplets were mostly 0.2 to 0.4 μm in diameter but could be as large as 1.0 μm. Many fibres also contained subsarcolemmal aggregates of mitochondria (fig. 3). Many of these mitochondria, along with those situated internally between the myofibrils, contained paracrystalline inclusions. At higher magnification the inclusions could be identified as type 1 (Stadhouders, 1981), with subunits formed by 4 parallel laminae located in the expanded space between the inner and outer mitochondrial membranes (Schmalbruch, 1983). The appearance of these subunits varied considerably depending on the direction in which they had been sectioned (fig. 3). The areas of mitochondrial abnormality contained high concentrations of glycogen.

After riboflavin treatment the numbers of lipid droplets were decreased to

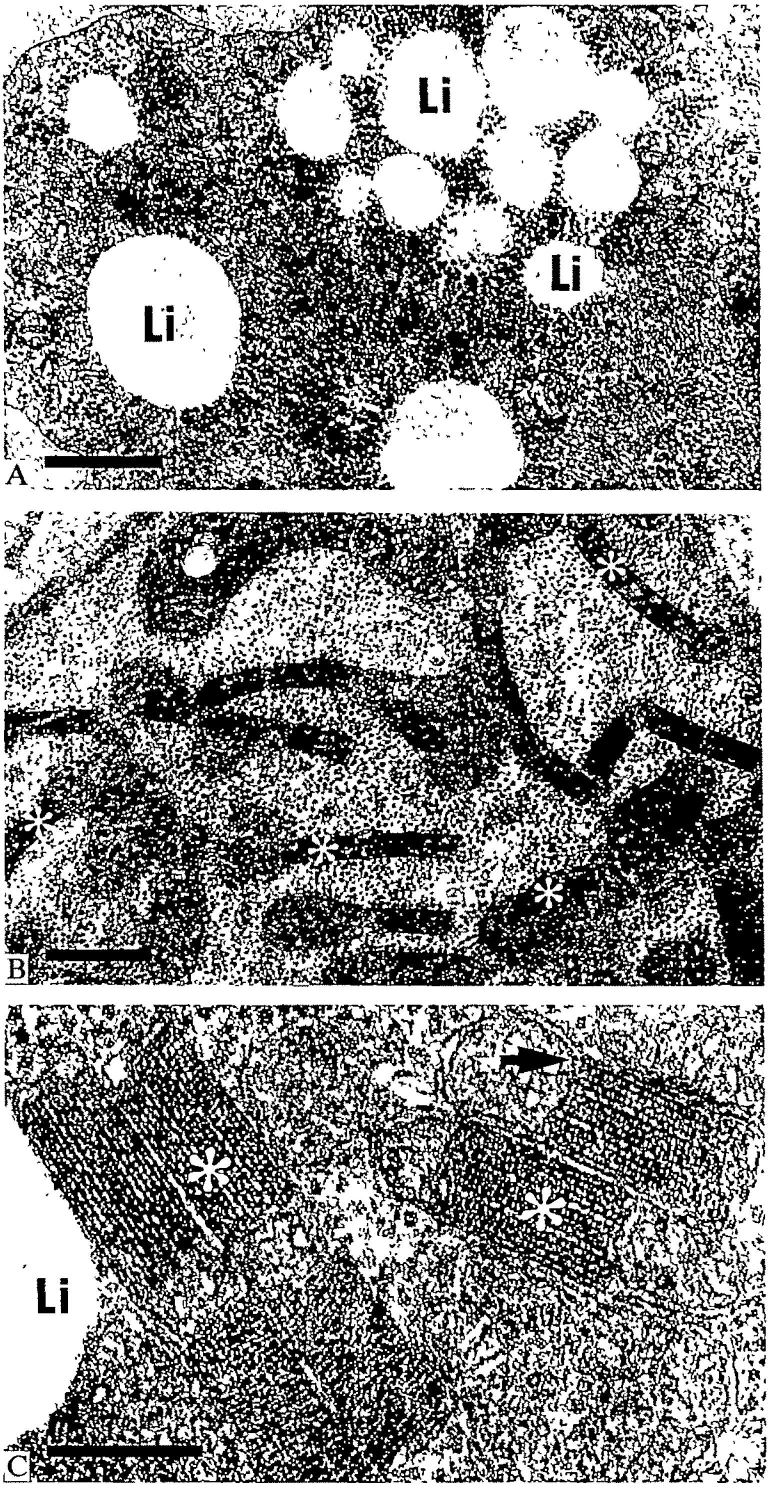

Fig 3 Pretreatment biopsy, electron microscopy A, an aggregation of lipid droplets (Li) at the periphery of a myofibre Bar = 0 5 μm. B, an aggregation of mitochondria, most of which contain paracrystalline arrays (asterisks). Note the large amounts of glycogen in this and preceeding electron micrograph. Bar = 0 5 μm. C, at higher magnification the 4 parallel laminae of the subunits of the paracrystalline inclusions can be distinguished Note that the inclusions lie outside the inner mitochondrial membrane (arrowhead). Bar = 0 2 μm.

TABLE 2 OXIDATION OF CITRIC ACID CYCLE INTERMEDIATES BY SKELETAL MUSCLE MITOCHONDRIA

	Patient (1985)	*Controls*
10 mM succinate oxidation	550	517±117
10 mM glutamate and 1 mM malate oxidation	280	252±18

Results are nmol of ferricyanide reduced · min^{-1} · mg $protein^{-1}$ Control values are mean ±SD for 13 normal subjects.

normal levels. Mitochondrial aggregates were less common and far fewer contained paracrystalline arrays, although these could still be found.

Mitochondrial enzyme activities

The flow of electrons from NAD^+-linked substrates and from succinate to ferricyanide was not impaired in the original biopsy, indicating that there is no defect of complexes I, II and III of the respiratory chain (Table 2). The cytochrome spectra were entirely normal (fig. 4).

The acyl-CoA dehydrogenase activities in the initial mitochondrial fraction were assayed spectrophotometrically and very low activities of all 3 acyl-CoA dehydrogenases were detected (Table 3). The mitochondrial fractions prepared from 2 controls and the patient's first muscle biopsy were stored at −20° C and assayed 12 months later using an ETF-linked assay when similar results were found (Table 4). Measurement of the activities of the short-chain and long-chain enzymes in freshly prepared mitochondria from the second muscle biopsy showed that these had increased markedly following administration of riboflavin to the patient (Table 5). The activity of medium-chain acyl-CoA dehydrogenase was low compared with our mean control value but there was considerable variability

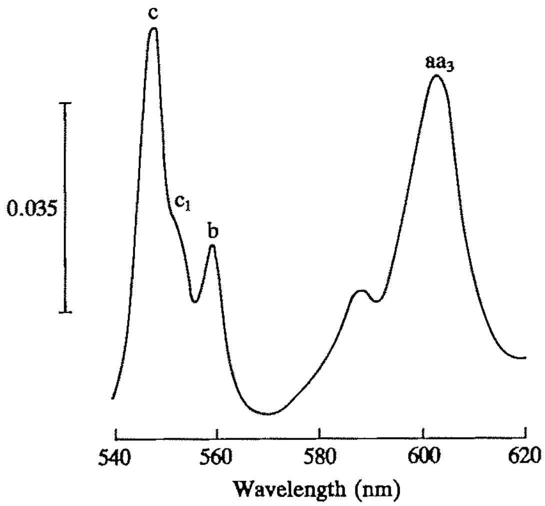

FIG. 4. Cytochrome spectrum of skeletal muscle mitochondrial fraction recorded at −190° C; sample cell reduced with 10 mM succinate in the presence of 0.1 mM KCN

TABLE 3. ACYL-CoA DEHYDROGENASES SPECTROPHOTOMETRIC ASSAY

	Patient (1985)	*Controls*	*Control activity* (%)
Butyryl-CoA (C_4)	1.7	8.5±3.1	20
Octanoyl-CoA (C_8)	1.3	9.2±1.1	14
Palmitoyl-CoA (C_{16})	13.7	23.7±6.1	58

Results are nmol of dichlorophenolindophenol reduced · min^{-1} · mg $protein^{-1}$. Control values are mean ±SD for 4 normal subjects

TABLE 4. ACYL-CoA DEHYDROGENASES. ETF ASSAY

	Patient (1985)	*Controls*	*Control activity* (%)
Butyryl-CoA (C_4)	0.7	4.3 (3.5–5.2)	16
Octanoyl-CoA (C_8)	1.4	8.8 (7.1–10.5)	16
Palmitoyl-CoA (C_{16})	1.7	4.1 (3.6–4.5)	42

Results are nmol of ETF reduced · min^{-1} · mg $protein^{-1}$ Control values are mean plus range for 2 normal subjects.

TABLE 5. ACYL-CoA DEHYDROGENASES· ETF ASSAY

	Patient (1986)	*Controls*	*Control activity* (%)
Butyryl-CoA (C_4)	16	15 (10–21)	106
Octanoyl-CoA (C_8)	20	60 (19–114)	33
Palmitoyl-CoA (C_{16})	52	53 (39–72)	98

Results are nmol of ETF reduced · min^{-1} mg $protein^{-1}$ Control values are mean ± range for 3 normal subjects

between our controls (Table 5). We have repeated the acyl-CoA dehydrogenase assays on the control and patient mitochondrial fractions and obtained similar results. The reason for the marked variability in the medium-chain acyl-CoA dehydrogenase activity in control mitochondrial fractions is not known. The difference in the mean control activity for all 3 acyl-CoA dehydrogenases from the first and second biopsies (Tables 4, 5) was due to the prolonged storage of the first biopsy sample at −20° C in the absence of protease inhibitors (I. M. Shepherd and S. Jackson, unpublished results).

Immunoblot analysis

There was little immunoreactive short-chain and medium-chain acyl-CoA dehydrogenase enzyme proteins in the mitochondrial fraction from the patient's first muscle biopsy (1985) as compared with normal controls (fig. 5). Immunoreactive proteins were present in similar quantities to controls in the mitochondria prepared from the second muscle biopsy after the patient had been treated

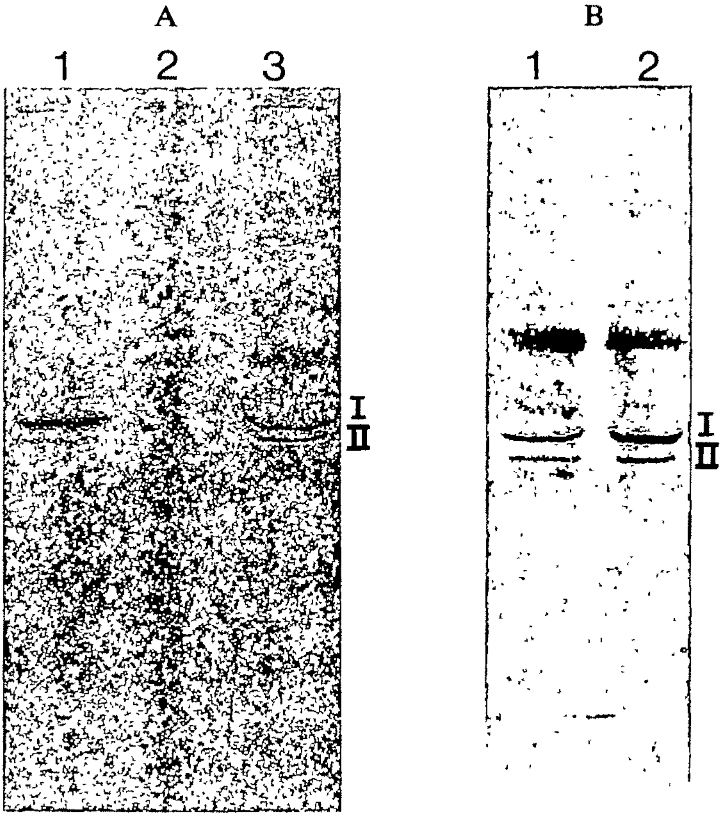

FIG. 5 Immunoblot analysis of acyl-CoA dehydrogenases in human skeletal muscle mitochondria. Human skeletal muscle mitochondrial proteins (100 μg of protein) were separated by SDS-polyacrylamide gel electrophoresis and immunoblotted using antibodies to short-chain and medium-chain acyl-CoA dehydrogenases. A, lanes 1 and 3, control human skeletal muscle mitochondria, lane 2, skeletal muscle mitochondria from patient (before treatment). All three mitochondrial fractions had been stored at −20° C for a similar period of time. B, lane 1, skeletal muscle mitochondria from patient (after treatment); lane 2, control human skeletal muscle mitochondria. I, medium-chain acyl-CoA dehydrogenase; II, short-chain acyl-CoA dehydrogenase.

with riboflavin. Antibodies were not prepared against long-chain acyl-CoA dehydrogenase and therefore the long-chain acyl-CoA dehydrogenase in the patient's mitochondria could not be studied by immunoblotting.

DISCUSSION

An increasing number of abnormalities of fatty acid oxidation are being recognized. We report a defect involving all 3 acyl-CoA dehydrogenases. This case presented as a painful proximal myopathy, which has partly improved after treatment with riboflavin.

Fatty acids are important substrates for both resting and exercising skeletal muscle (Felig and Wahren, 1975). Many defects of mitochondrial fatty acid oxidation may theoretically occur (Turnbull *et al.*, 1987), although the commonest so far found are defects of the acyl-CoA dehydrogenases. The straight-chain acyl-CoA dehydrogenases catalyse the dehydrogenation of acyl-CoA to enoyl-CoA in the first step of each cycle of β-oxidation. There is evidence that skeletal muscle contains 3 acyl-CoA dehydrogenases (short-chain, medium-chain and long-chain) (I. M. Shepherd, unpublished observations, 1987) similarly to liver and heart. All 3 acyl-CoA dehydrogenases from bovine heart and liver have molecular weights of approximately 180000 Da and are composed of 4, possibly identical, subunits. They have tightly, but noncovalently bound flavin adenine nucleotide (FAD) as prosthetic groups. These 3 enzymes have different but overlapping chain-length specificities (*see* Schulz, 1985). In cultured human fibroblasts about 50% of activity with butyryl-CoA is due to medium-chain acyl-CoA dehydrogenase (Coates *et al.*, 1986). No systematic studies of the specificity of the human enzymes have yet been made.

Acyl-CoA dehydrogenase deficiency may present in a variety of different ways. Four patients with a deficiency of short-chain acyl-CoA dehydrogenase have been described. Turnbull *et al.* (1984) described a 46-yr-old woman with proximal muscle weakness and muscle pain. She had a lipid storage myopathy and a low activity of dehydrogenation of short-chain acyl-CoA esters in muscle. Short-chain acyl-CoA dehydrogenase activity in cultured fibroblasts from this patient was normal (Turnbull *et al.*, 1984) and there was a large ketotic response to fasting, indicating that the liver enzyme was also normal. Coates *et al.* (1986) described an infant who presented with failure to thrive, hypotonia, developmental delay, progressive muscle weakness and microcephaly. Short-chain acyl-CoA dehydrogenase was defective in cultured fibroblasts from this patient. Amendt *et al.* (1987) have recently described 2 children with short-chain acyl-CoA dehydrogenase deficiency. The first presented with a severe metabolic acidosis following which she has grown and developed normally. The second child died on the sixth day of life with hypotonia, coma and a metabolic acidosis. The activity of short-chain acyl-CoA dehydrogenase was low in cultured skin fibroblasts from both these patients.

Medium-chain acyl-CoA dehydrogenase deficiency presents in various ways including an acute fulminating illness (Duran *et al.*, 1986), sudden infant death syndrome (Howat *et al.*, 1985), with features like Reye's syndrome (Coates *et al.*, 1985), unexplained hypoglycaemia (Coates *et al.*, 1985) and systemic carnitine deficiency (Zierz *et al.*, 1986). Some family members of children that have died from medium-chain acyl-CoA dehydrogenase deficiency are asymptomatic although β-oxidation of medium-chain fatty acids in fibroblasts was impaired (Duran *et al.*, 1986). In many patients muscle symptoms are not prominent, but others have muscle weakness (Glasgow *et al.*, 1980). The reason for the clinical heterogeneity is not known.

Long-chain acyl-CoA dehydrogenase deficiency has only been reported in a few cases. Hale *et al.* (1985) described 3 children who presented in early childhood with hypoglycaemia, hepatomegaly, cardiomyopathy and muscle weakness. Naylor *et al.* (1980) reported 2 siblings with intermittent hypoglycaemia, lethargy and coma who were subsequently shown to have long-chain acyl-CoA dehydrogenase deficiency. Muscle symptoms were not prominent in these last 2 children.

Another condition which is associated with impaired fatty acid oxidation is glutaric aciduria type II or multiple acyl-CoA dehydrogenase deficiency. The underlying biochemical abnormality in this condition is deficiency of electron transfer flavoprotein (ETF) or electron transfer flavoprotein : ubiquinone oxidoreductase (ETF : QO) (Frerman and Goodman, 1985*b*; Ikeda *et al.*, 1986). The activity of the acyl-CoA dehydrogenases is normal. ETF and ETF : QO mediate the transfer of electrons from the acyl-CoA dehydrogenases to the respiratory chain. The majority of patients with glutaric aciduria type II present in infancy with severe systemic symptoms (Goodman and Frerman, 1984), but a few patients have presented with predominantly muscle symptoms (de Visser *et al.*, 1986; Di Donato *et al.*, 1986). Treatment with pharmacological doses of riboflavin seems to have been effective in some cases of glutaric aciduria type II (de Visser *et al.*, 1986).

The patient described in this paper has low activity for the dehydrogenation of all straight-chain acyl-CoA esters. In addition, less immunoreactive medium-chain and short-chain acyl-CoA dehydrogenase enzyme proteins were found in the first muscle biopsy compared with controls. This man's clinical picture is similar to that of a woman with short-chain acyl-CoA dehydrogenase deficiency (Turnbull *et al.*, 1984). In both cases the liver does not appear to have been involved as there were marked ketogenic responses to fasting before treatment with riboflavin (Table 1), indicating an adaptive increase in the utilization of ketone bodies by peripheral tissues. This is consistent with the occurrence of different isoenzymes of acyl-CoA dehydrogenases in muscle and in liver and possibly other tissues.

The cause of the abnormality of β-oxidation is unknown. It could be due to a genetic mechanism causing a failure in enzyme synthesis or increasing catabolism with late onset, or to an unknown environmental factor. There appear to be quantitative rather than qualitative changes in short-chain and medium-chain acyl-CoA dehydrogenases and presumably also in the long-chain acyl-CoA dehydrogenase. It is difficult to explain the improvement following administration of pharmacological doses of riboflavin, if this is not a coincidence, in the absence of apparent riboflavin deficiency. It is not known how the concentrations of FAD in tissues change following treatment with riboflavin. Possible sites of the defect include impaired cellular uptake of riboflavin, FAD synthesis, FAD transport into mitochondria or the binding of FAD to the apoenzymes. Riboflavin or FAD could influence the turnover of enzymes by increasing their rate of synthesis, for example increasing the rate of transcription of the DNA coding them, or decreasing their degradation by stabilizing the enzymes in some way. Other vitamins may stimulate the activity of their dependent enzymes as has been reported with the increased activity of biotin-dependent enzymes in human leucocytes by the administration of large doses of biotin (Bartlett *et al.*, 1980). At present there is no way of deciding which of the above possibilities applies to our patient.

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THE CLINICAL SPECTRUM OF ALCOHOLIC PELLAGRA ENCEPHALOPATHY

A RETROSPECTIVE ANALYSIS OF 22 CASES STUDIED PATHOLOGICALLY

by MICHEL SERDARU,[1] CHANTAL HAUSSER-HAUW,[2]
DOMINIQUE LAPLANE,[3] ANDRÉ BUGE,[4] PAUL CASTAIGNE,[3]
MAURICE GOULON,[5] FRANÇOIS LHERMITTE[1]
and JEAN-JACQUES HAUW[2]

(*From the* [1]*Clinique de Neurologie et de Neuropsychologie, Hôpital de la Salpêtrière, Université Paris VI, the* [2]*Laboratoire de Neuropathologie Raymond Escourolle, Hôpital de la Salpêtrière, the* [3]*Clinique des Maladies du Système Nerveux, Hôpital de la Salpêtrière, the* [4]*Clinique de Neurologie, Hôpital de la Salpêtrière and the* [5]*Clinique de Réanimation, Hôpital Raymond Poincaré, Garches, France*)

SUMMARY

A retrospective clinical study of 22 heavy alcohol drinkers is reported in which postmortem study showed diffuse chromatolysis of neurons identical to that found in neurological pellagra, associated in 13 cases with Marchiafava-Bignami disease and/or Wernicke-Korsakoff disease. The clinical features included confusion and/or clouding of consciousness, marked oppositional hypertonus ('gegenhalten') and myoclonus. Because of the frequent coexistence of other alcoholic encephalopathies in the same patient, alcoholic pellagra was often unrecognized. Fifteen patients received thiamine and pyridoxine therapy without niacin. It appeared to aggravate the neurological state or to trigger the development of alcoholic pellagra encephalopathy in 9 cases. The relationship between pellagra occurring during thiamine and pyridoxine therapy and 'nicotinic acid deficiency' is discussed. Multiple vitamin therapy should be given in the treatment of undiagnosed encephalopathies in alcoholic patients.

INTRODUCTION

Endemic pellagra has disappeared from developed countries but 'secondary pellagra' still occurs, related to disorders or to treatments which interfere with tryptophan availability or metabolism. It has been described in association with many disorders of the alimentary tract (Pallis and Lewis, 1974), as well as with malignant carcinoid, and the administration of isonicotinic acid, thiosemicarbazone, isoniazid, 6-mercaptopurine, 5-fluorouracil or puromycin (Still, 1976). The term of 'alcoholic pellagra' or 'pseudopellagra' was first used by Leudet in

Correspondence to Dr Jean-Jacques Hauw, Laboratoire de Neuropathologie Raymond Escourolle, Hôpital de la Salpêtrière, 47 Bld de l'Hôpital, 75651 Paris Cedex 13, France

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1869. This author reported 5 alcoholic patients with signs and symptoms similar to those seen in pellagra, namely dermatitis, diarrhoea and dementia. Meyer (1901) described 2 cases of 'alcoholic senile deterioration' (Cases 5 and 8) suffering from weakness, delirium, stupor or coma and 'jerking of all muscles'. The unique neuropathological finding was neuronal chromatolysis, but Meyer did not mention the term pellagra in this paper. The association of chronic alcoholism and pellagra was later stressed by Klauder and Winkelman (1928), Bender and Schilder (1933), Spies and DeWolf (1933), Zimmerman *et al.* (1934), Schwob *et al.* (1953), Gruner (1956), Deparis *et al.* (1961), Still (1976), Spivak and Jackson (1977), Ishii and Nishihara (1981), Lishman (1981) and Serdaru *et al.* (1981), among others. Alcoholic pellagra is thought to be due to a deficiency of niacin and possibly other B group vitamins (Jolliffe *et al.*, 1940; Zieve and Hill, 1963; Barrett-Connor, 1967; Spivak and Jackson, 1977; Ishii and Nishihara, 1981). However, the precise mechanism of this disorder still needs to be established. The progressive development of encephalopathy in alcoholic patients submitted to thiamine and pyridoxine therapy without niacin raises the question of a possible relationship with the 'nicotinic acid deficiency syndrome' described by Jolliffe *et al.* (1940), who considered alcoholic pellagra encephalopathy as a 'complete nicotinic acid deficiency' which developed so rapidly that changes in mouth and skin characteristic of pellagra do not have time to develop.

The systematic postmortem study of alcoholic encephalopathies at the Laboratoire Raymond Escourolle from 1952 to 1983 led to the diagnosis of alcoholic pellagra in 22 cases (for details, *see* Hauw *et al.*, 1988). The retrospective analysis of the case records of these patients allowed us to define the clinical features associated with alcoholic pellagra.

CASES

Depending on the pathological findings, we divided the 22 patients into 2 groups (Table 1): (1) isolated changes of alcoholic pellagra; (2) alcoholic pellagra associated with other alcoholic encephalopathies, such as Marchiafava-Bignami disease and/or Wernicke-Korsakoff disease. Nine of these cases have been reported elsewhere: Case 6 by Serdaru *et al.* (1981) and Cases 10 and 12–16 by Castaigne *et al.* (1971) as Cases 2, 6, 7, 4, 5 and 3, respectively. Case 17 has been reported by Haguenau *et al.* (1955) and Case 22 by Serdaru *et al.* (1982).

RESULTS

The age and sex of the patients are shown in Table 1. All patients were heavy wine drinkers except Case 14 who drank mainly whisky. Most of them were neglected; 9 were undernourished and cachectic. Several belonged to the classical category of French tramps with no fixed abode ('clochards'). Clinical signs appeared progressively (over weeks) or abruptly (in a few hours or days), before or during hospitalization. The main clinical features are given in Table 2. Three clinical signs were frequently associated in our patients: a fluctuating confusional

TABLE 1. DISTRIBUTION OF THE 22 CASES BASED ON THE PATHOLOGICAL FINDINGS

Groups	*Number*	*Male*	*Female*	*Age (yrs)*
I. Pseudopellagra (PP)	9	6	3	43-64
II. PP and MBD	8	7	1	39-59
PP and WKD	4	1	3	43-66
PP, MBD and WKD	1	0	1	54
Total	22	14	8	

MBD = Marchiafava-Bignami Disease; WKD = Wernicke-Korsakoff disease.

state and/or clouding of consciousness (22/22 cases), oppositional hypertonus ('gegenhalten') (22/22) and startle myoclonus (12/22).

Marked hypertonus was the most striking feature. It was of the 'oppositional' type, always involving the limbs and less often the trunk and abdominal muscles. In 10 patients, the neck was not involved although other muscle groups were severely hypertonic. The neck was stated to be involved in only 2 patients; in the other patients, involvement of the neck was not specified. Passive activation of a remote muscle group was immediately followed by an hypertonic reinforcement of the examined segment. No postural reflexes were elicited. Myoclonic jerks were observed in most but not in all patients. Noise and cutaneous stimulation facilitated their occurrence, but intention and action had no obvious effect. The limbs were the most involved part of the body. At times, twitching of the face was noticed. Velopalatine myoclonus was never observed. A fluctuating confusional

TABLE 2. CLINICAL DATA FOR THE 22 PATIENTS, GROUPED ACCORDING TO THE PATHOLOGICAL FINDINGS

Groups	*PP (9 cases)*	*PP+MBD (8 cases)*	*PP+WKD (4 cases)*	*PP+WKD+MBD (1 case)*
Confusion	8/8	6/7	4/4	1
Grasping-groping	1/9	3/7	1/4	1
Hypertonus	9/9	8/8	4/4	1
Trismus	1/9	2/8	0	0
Paroxysmal reinforcement of hypertonus	0	3/8	0	0
Myoclonus (± unspecified abnormal movements)	4±2/9	2/8	3/4	1
Lateralized motor signs	0/9	5/8	0/4	1
Oculomotor disturbances	0/9	2/8	3/4	1
Cerebellar signs	2/9	3/7	0	0
Dysarthria	6/9	6/7	1/4	?
Terminal coma	7/8	6/8	4/4	1
Cachexia	5/9	4/8	0/4	0
Dermatitis	1/9	0/8	1/4	0
EEG slowing	7/7	6/6	3/3	?

PP = alcoholic pellagra; MBD = Marchiafava-Bignami disease; WKD = Wernicke-Korsakoff disease. 0 = absent; ? = not known.

state was frequent. Global memory loss, visual hallucinations and restlessness alternating with apathy were the most frequent alterations of mentation. Clouding of consciousness, ranging from stupor to coma, appeared sooner or later in every case. In patients whose survival and level of consciousness allowed a thorough examination, the mental symptoms appeared to fluctuate throughout the day. At times these patients had normal behaviour and mentation and even expressed complaints about their mental and physical health. Skin manifestations consisted of erythematous lesions on the back (Case 1), and on the chest (Case 21). Polyneuropathy was recorded 4 times (Cases 3, 17, 18 and 20) but nerve conduction studies were not performed in any of the patients. Other disturbances that occurred in 5 patients included hyperthermia in Cases 11 and 14, hypothermia in Case 13, vomiting in Case 14 and anisocoria in Case 17.

Common metabolic encephalopathies (hepatic, disturbed water and electrolyte balance, hypoglycaemic, hypercalcaemic, hypocalcaemic) were excluded by normal laboratory tests in all except 2 cases. CSF examination, when performed, eliminated meningitis and subarachnoid haemorrhage.

Electroencephalography was always abnormal, even when consciousness was reported to be normal. Every available EEG showed bilateral slow wave activity, mainly in the theta range. No pseudoperiodic slow activity or epileptiform discharges were observed.

The disease was considered to be of 'primary onset' when some clinical signs, at least hypertonus, were maximal from the time of admission (13 cases). A catastrophic course with death before the eighth day occurred in 8 of these cases, 4 of whom had received thiamine and pyridoxine therapy. A longer course was observed in 5 cases who did not receive such therapy. The disease was considered to be of 'late onset' when hypertonus, absent on admission, developed later, or when hypertonus, present on admission, subsequently increased markedly (9 cases). The delay was always 5 days or longer (5–60 days). All the patients who displayed late onset signs of pellagra had been clinically diagnosed as alcoholic encephalopathy, delirium tremens or Wernicke-Korsakoff disease and thus had received thiamine and pyridoxine therapy from admission. Details of treatment with thiamine and pyridoxine are shown in Table 3. In these 9 cases, the appearance of neurological signs was related to the thiamine and pyridoxine therapy. This was statistically significant ($\chi^2 = 7.1$, $P = 0.0007$).

ILLUSTRATIVE CASES

Case 1

M.C., a 57-yr-old man, was admitted because of transient loss of consciousness. On intitial examination, he was dirty, covered with lice and poorly nourished. He responded only to simple commands which had to be repeated several times. From time to time, he was able to remember that he had fallen down and sustained a slight head injury. Severe oppositional hypertonus was the outstanding sign. The arms were fixed in flexion, the legs hyperextended. The hypertonus also involved the neck and abdominal muscles. The tendon reflexes were brisk and the plantar responses

TABLE 3 LENGTH OF SURVIVAL FOR THE 22 CASES AS A FUNCTION OF VITAMIN THERAPY AND PATHOLOGICAL FINDINGS

Groups	*Type of evolution*	*(n)*	*Mean disease duration (days)*	*Range of disease duration (days)*	*Vitamin B therapy* *n*	*Vitamin B therapy* *(Cases)*	*No Vitamins* *n*	*No Vitamins* *(Cases)*
Alcoholic pellagra (PP)	P	(6)	20	4–30	3	(1, 2, 9)	3	(3, 5, 8)
	L	(3)	42	26–74	3	(4, 6, 7)		0
PP and MBD	P	(5)	6	2–10	2	(12, 17)	3	(10, 11, 14)
	L	(3)	45	12–78	3	(13, 15, 16)		0
PP and WKD	P	(1)	4	4	1	(21)		0
	L	(3)	72	13–120	3	(18, 19, 20)		0
PP, WKD and MBD	P	(1)	3	3	0		1	(22)
	L	(0)	—	—	0			0
Total	P	(13)			6*			7*
	L	(9)			9*			0*

PP = alcoholic pellagra, MBD = Marchiafava-Bignami disease; WKD = Wernicke-Korsakoff disease; P = primary onset; L = late onset; n = number of cases; * = χ^2 7.1077, $P = 0.0007$.

flexor. The skin showed erythematous lesions, mainly on the back. The CSF was normal as were routine biological investigations. Intramuscular vitamin B therapy was started (thiamine 1 g, pyridoxine 0.5 g, daily). On the morning of the third day, 'abnormal movements' were noted. In the evening, the patient became deeply comatose and died 4 days after admission from acute pulmonary oedema. Pathological findings: pellagra.

Case 4

C.F., a 52-yr-old man, was admitted because of weakness and weight loss. Initial clinical examination was considered within normal limits. He was given i.m. vitamin B therapy (thiamine 0.5 g, pyridoxine 0.5 g, daily) because of his drinking habits. His condition worsened gradually and he developed confusion and hypertonus. On reevaluation (twentieth day), he was slightly obtunded and was only able to stutter a few words. Oppositional hypertonus of the limbs and startle myoclonus of the limbs and face were recorded. The tendon reflexes were brisk and the right plantar reflex extensor. No grasp reflex was observed. Biochemical studies, including CSF and blood ammonia, were normal. A right carotid angiogram was normal. EEG showed a diffuse slow activity. The level of consciousness deteriorated and the patient died from aspiration pneumonia 26 days after admission. Pathological findings: pellagra.

Case 6

R.C., a 52-yr-old man, was admitted because of rapidly evolving ataxia of gait. Two days later, he developed a confusional state, diagnosed as delirium tremens. Rehydration and i.m. vitamin B therapy (thiamine 0.5 g, pyridoxine 0.25 g, daily) induced a measurable improvement in the ataxia and confusion. Thirty-five days after admission, despite continued parenteral vitamin B therapy, his gait again started to deteriorate. On neurological examination, he showed fluctuating mental disturbances and agraphia. Marked oppositional hypertonus was observed, involving the limbs more than the axial muscles and sparing the neck. Irregular asynchronous startle myoclonus was observed. The tendon reflexes were brisk and the plantar reflexes nonreactive. There was a bilateral groping reflex. Biochemical examination, including CSF, blood ammonia and pyruvate, were within normal limits. EEG showed diffuse slow activity. Kept on the same treatment, the patient deteriorated rapidly; he showed further swallowing disturbances, weight loss, dysarthria and ultimately stupor and coma. He died from aspiration pneumonia 74 days after admission. Pathological findings: pellagra.

Case 11

Z.R., a 39-yr-old man, was admitted in coma. He was undernourished and hyperthermic. Examination showed oppositional hypertonus and bilateral extensor plantar responses. Upgaze was impaired. During the next few days, several episodes of paroxysmal increase in the hypertonus were observed. CSF and blood analyses (not including pyruvate and blood ammonia levels) were normal. The EEG showed diffuse nonspecific slow activity in the theta and delta range. The patient did not receive any vitamin B therapy. He died suddenly, 1 week after admission. Pathological findings: pellagra and Marchiafava-Bignami disease.

Case 19

R.S., a 53-yr-old woman whose past medical history included several episodes of delirium tremens, was admitted in a fluctuating confusional state. On examination, her level of consciousness appeared to vary. When able to respond, she could answer only simple questions. Memory impairment and confabulation were noted. Bilateral ptosis, bilateral extensor plantar responses and bilateral grasp reflexes were documented. Irregular and infrequent myoclonic jerks were seen. There was a marked oppositional hypertonus of the limbs and trunk, sparing the neck. The EEG showed diffuse slow activity. The patient was given i.m. vitamin B therapy (thiamine 1 g; pyridoxine 0.5 g, daily). Some improvement was observed, her level of consciousness and memory returning to normal after 5 days. During the next few days, however, the hypertonus increased sharply (still sparing the neck). The patient became comatose and died on the thirteenth day after admission. Pathological findings: pellagra and Wernicke-Korsakoff disease.

Case 22

Q.G., a 54-yr-old left-handed woman, was admitted with mutism and akinesia. On initial examination, her level of consciousness was normal. Absence of activity of her right side and deviation of the head and eyes to the left were attributed to right motor and sensory neglect. There was marked bilateral hypertonus, sparing the neck, which was more pronounced on the right. Startle myoclonus of the right arm and leg was observed. The tendon reflexes were brisk, the plantar responses extensor and there was a left grasp reflex. Biochemical studies, including CSF, were normal. A left carotid angiogram was normal. An EEG was not performed. No vitamin B therapy was given. The patient died 3 days after admission. Pathological findings: pellagra, Wernicke-Korsakoff disease and Marchiafava-Bignami disease.

DISCUSSION

Nonendemic pellagra is usually not characterized by the classical triad, as dermatitis and diarrhoea are often lacking. This is the case in pellagra secondary to malabsorption (Tabaqchali and Pallis, 1970), to isoniazid administration (Ishii and Nishihara, 1985) or to alcoholism (Klauder and Winkelman, 1928; Zimmerman *et al.*, 1934; Jolliffe *et al.*, 1940; Spillane, 1947; Spivak and Jackson, 1977; Ishii and Nishihara, 1981). The diagnosis, which can only be suspected on clinical grounds, is confirmed by a response to niacin or by postmortem neuropathological study. Two of the 3 cases reported by Zimmerman *et al.* (1934) had only neurological signs. Jolliffe *et al.* (1940) stated that 'patients showing the pellagra encephalopathic syndrome . . . without signs of (skin) pellagra, would represent a complete nicotinic acid deficiency which develops so rapidly that the structural changes in the mouth and skin characteristic of pellagra do not have time to occur'. Spivak and Jackson (1977) reported only 2 'full triad pellagra' among their 18 alcoholic pellagrins, although all their patients had some degree of dermatitis. The rarity of sunlight in Paris could explain the scarcity of skin pellagra. Indeed, Klauder and Winkelman (1928) and Spivak and Jackson (1977) described a seasonal variation in the occurrence of pellagrous encephalopathy in their alcoholic patients.

Our patients were all heavy alcoholic drinkers, and used to drink mainly red wine, which is a poor source of niacin (Barrett-Connor, 1967). Nevertheless, Zimmerman *et al.* (1934) stated 'It is a curious fact that such a deficiency disease as pellagra tends to appear in people who drink distilled liquors but rarely in those who drink wine or beer'. Meyer (1901) and Spies and DeWolf (1933) considered that whisky predisposes to pellagra. The frequency of alcoholic pellagra in France might be explained by the prevalence of red wine drinkers and the absence of addition of niacin to alimentary products. Moreover, in this country parenteral thiamine and pyridoxine therapy is usually preferred to multivitamin treatment for alcoholic encephalopathy on admission to the emergency room.

The most frequent clinical signs encountered in our patients were confusion and/or clouding of consciousness (22/22 cases), oppositional hypertonus (22/22) and myoclonic jerks (12/22). Confusion, alternating with short periods of normal mentation, was always present in the noncomatose patients. It was accompanied by restlessness, disorientation, visual hallucinations and gross memory loss. Confusional states and clouding of consciousness, always fluctuating, are reported in all the papers dealing with alcoholic pellagra. Klauder and Winkelman (1928), in their group of 'grave pellagra', described a 'complete disorientation and delirium'. Spivak and Jackson (1977) stated that when neurological disturbances exist, there is disorientation, particularly for time, and impairment of recent memory. Ishii and Nishihara (1981) mentioned mental disturbances in all their cases. The causes of confusion and/or an altered state of consciousness are numerous. They are the hallmark of metabolic encephalopathies. Thus this sign of alcoholic pellagra encephalopathy is rather nonspecific. The mental condition in the terminal episode—apart from confusion—was not typically that of anxious, perplexed agitation described in endemic pellagra since the early descriptions (Landouzy, 1862; Spies *et al.*, 1938), in a case of endogenous pellagra without hyperaminoaciduria (Goulon *et al.*, 1969) and in a few reports of alcoholic pellagrous encephalopathy (Ishii and Nishihara, 1981). In our series, it was more that of profound apathy alternating with restlessness. The same state was described by Meyer (1901).

Hypertonus of the oppositional type, usually sparing the neck, was found in all our patients. It was similar to 'gegenhalten' (counterfixation), as described by Kleist (1927) in association with thalamic strokes. This author defined 'gegenhalten' as a resistance to change in posture brought about by muscle tension which increases with the effort applied by the examiner. It is obviously different from spasticity. Bender and Schilder (1933) quoted him, when referring to the 'special type of changing rigidity in the limbs and neck' that they observed in several of their alcoholic patients suffering from encephalopathies, one of which (Case 12) had some chromatolytic changes. Schwob *et al.* (1953) described the 'peculiar generalized muscular contracture' in 2 cases of pathologically proven pellagra. Typical oppositional hypertonus may occur in various metabolic encephalopathies, the most common being hepatic encephalopathy in which the neck is usually involved. Plum and Posner (1972) found 'gegenhalten' rigidity in coma and mostly in metabolic coma. In our series, gegenhalten was usually observed days or weeks before the development of coma.

The anatomical substratum of oppositional hypertonus is unknown. It is usually not related clinically to disturbances of the pyramidal system. On neuropathological examination, it was often little affected in our series, neither were the thalami. The basal ganglia (caudate and subthalamic nuclei, pallidum, putamen) and substantia nigra are also mostly spared in alcoholic pellagra. Involvement of the cells of the reticular formation has suggested a possible role for this structure in oppositional hypertonus since the description of Bender and Schilder (1933). Little is known about 'reticular hypertonus', although a few reports dealing with degenerative disorders of the reticular formation defined rigidity as the main clinical feature (Verhaart, 1958; Martinez-Lage *et al.*, 1972). Marked rigidity is induced in monkeys by tegmental lesions extending up to the rostral reticular formation (Poirier, 1960). In our series, these latter areas were markedly affected. The pontine nuclei are also heavily involved in alcoholic pellagra, which made Gruner (1956) think that lesions of these structures may be responsible for the marked hypertonus found in alcoholic pellagrins. In some patients with prominent hypertonus, only the pontine nuclei were involved. Thus the peculiar oppositional hypertonus found in alcoholic pellagra may indeed bear some relationship to the pontine nuclei.

The third main clinical feature of alcoholic pellagra in our series was myoclonus (11/22 cases). 'Frequent attacks of involuntary jerking' (Turner, 1899), 'abnormal jactitations of the limbs' (Meyer, 1901), 'spasmodic jerking' (Klauder and Winkelman, 1928), 'myoclonic jerks' (Bender and Schilder, 1933) and unspecified abnormal movements or 'muscle twitchings' (Zimmerman *et al.*, 1934; Gruner, 1956) have been described. Multifocal myoclonus is seen in a number of metabolic brain diseases. The movements are most common in the face and shoulders but may occur anywhere in the body. They occur at a later and more severe stage of metabolic illness than does asterixis (Posner, 1975). However, alcoholic pellagra is hardly if ever thought of when myoclonus is observed (Fahn *et al.*, 1986). Various CNS structures have been implicated in the causation of myoclonus (Fahn *et al.*, 1986; Hauw *et al.*, 1986). In our series, the cerebral cortex was only mildly involved and the corticospinal tracts and basal ganglia were spared. The paucity of changes noted in the medullary olives and the importance of the clinical signs argue against a causal relationship between these lesions and myoclonus. However, the dentate nuclei, which were markedly involved, could account, at least in part, for the appearance of myoclonus in the patients of our series.

Other clinical features exhibited by our patients included ataxia, cerebellar signs, hyperreflexia, pyramidal signs, grasping and groping, opisthotonic crises, seizures, cranial nerve involvement, peripheral neuropathy and autonomic dysfunction. These signs are consistent with those reported in the main papers already quoted. However, Zimmerman *et al.* (1934) were the only authors to include 'opisthotonic spasms' (Case 3), in their description of alcoholic pellagra. In our series, opisthotonic crises were noticed in only 2 patients, who had Marchiafava-Bignami disease in addition to pellagra. Castaigne *et al.* (1971) reported 4 cases of pure Marchiafava-Bignami disease (excluding cases with pellagra), who displayed oppositional hypertonus. One of them showed episodes of 'paroxysmal tetanoid contracture'. Trismus, which may be considered as a focal tetanoid contracture, occurred in 3 patients of our series, 2 of them having pathologically proven Marchiafava-Bignami disease in addition to pellagra. Opisthotonic crises and trismus are therefore more likely to be related to Marchiafava-Bignami disease than to other alcoholic encephalopathies.

Seizures occurred in only 2 of our patients. EEGs did not show epileptiform activity in any of the cases. Nonspecific slow activity was recorded. In 29 adult endemic pellagrins, an excess of theta and occasionally delta activity was recorded in 22 cases (Srikantia *et al.*, 1968). Such abnormal EEGs could occur despite an apparently normal mental state, whereas the EEG was always abnormal when the patients had obvious clinical mental changes. The same excess of theta activity was reported by Still (1976) and Spivak and Jackson (1977) in the EEGs of alcoholic pellagrins. Clinical peripheral neuropathy was noticed in 5 cases (3, 9, 16, 17, 20). Seven of the 20 cases reported by Ishii and Nishihara (1981) had a neuropathy. In endemic pellagra, neuropathy may be more prevalent, up to 75% according to Lewy *et al.* (1940).

Pellagra had not been suspected clinically in our patients. In most cases, the signs and symptoms were incorrectly attributed to other acute or subacute alcoholic encephalopathies. The differential diagnosis of alcoholic pellagra encephalopathy includes alcohol withdrawal, hepatic encephalopathy, Wernicke-Korsakoff or Marchiafava-Bignami disease (Victor and Banker, 1978). The course of the disease would argue against a diagnosis of an alcohol withdrawal syndrome for every patient: in 16 cases, the symptoms lasted at least 7 days, which excluded a withdrawal syndrome. With regard to the 6 patients with an earlier fatal outcome, the onset of signs and symptoms antedated alcohol withdrawal. Nine patients showed the first neurological signs at least 5 days after admission. Hepatic encephalopathy occurs mainly in cirrhotic patients. In our series, the clinical signs did not include tremor and asterixis, and biochemical screening, when performed, was normal or only mildly abnormal. The EEG showed only mild nonspecific slow activity. Examination of the liver, available in 16 cases, differed from the marked hepatic disorders which are usually associated with chronic hepatic encephalopathy (Victor *et al.*, 1965).

Five patients showed changes of Wernicke-Korsakoff disease on neuropathological examination. One patient showed bilateral ptosis. Marchiafava-Bignami disease is a rare disorder. Eight of our patients showed histopathological signs of this disorder. The salient clinical finding in such cases occurring in association with pellagra was the occurrence of opisthotonic crises and/or trismus, as already discussed. Oppositional hypertonus was present in all these cases but in Cases 13, 15 and 16, it appeared only after thiamine and pyridoxine therapy—which seems unlikely to trigger Marchiafava-Bignami disease. Hypertonus has been described in this condition (Rancurel, 1966; Castaigne *et al.*, 1971; Victor and Banker, 1978), although its clinical features are quite variable.

The 3 main clinical features listed above, namely confusion or an altered state of consciousness, oppositional hypertonus and myoclonus, even if nonspecific when isolated, should evoke the possibility of pellagra encephalopathy if they occur together, more or less progressively, in an alcoholic patient. Alcoholic pellagra encephalopathy can be distinguished from other acute or subacute encephalopathies on clinical, biochemical and pathological grounds. However, in practice, because of the frequent coexistence of several different alcoholic encephalopathies in the same patient, it may be difficult to identify the precise diagnosis.

On the basis of the alcoholic background, thiamine and pyridoxine therapy was started in most patients. Unfortunately, niacin was never added. In the group who received i.m. therapy, 9 patients survived long enough to allow the appearance of secondary onset encephalopathy (Cases 4, 5, 7, 13, 15, 16, 18–20). During the first phase of treatment, the patients improved. Signs of pellagra encephalopathy appeared or increased for at least 5 days (5–60 days) after the beginning of treatment. Statistical analysis of the evolution of the encephalopathy in relation to the thiamine and pyridoxine therapy showed a significant correlation ($\chi^2 = 7.1$, $P = 0.0007$) between late-onset pellagra encephalopathy and thiamine and pyridoxine therapy. In most cases, it was difficult to know whether pellagra was present from the outset and worsened progressively, while associated alcoholic encephalopathies (hepatic encephalopathy, Wernicke-Korsakoff or Marchiafava-Bignami disease) improved, or whether thiamine and pyridoxine therapy, while improving associated alcoholic encephalopathies, precipitated the onset of pellagra encephalopathy. However, Case 4 had no neurological signs on admission. He was given i.m. thiamine and pyridoxine because of weakness and weight loss. He developed pellagra encephalopathy. Antagonism between thiamine and niacin has already been suggested (Spies *et al.*, 1938; Lehmann and Nielsen, 1939; Lishman, 1981). The metabolic demands from a relative excess of thiamine may increase the requirements for pyridine coenzymes (NAD and NADP which require nicotinic acid as cofactor) and result in pellagra (Barrett-Connor, 1967). Multiple vitamin therapy, adopted as a routine from the 1950s onwards, is the probable explanation for the demise of the nicotinic acid deficiency syndrome in the UK and the USA (Lishman, 1981).

In this study, the patients with prolonged survival, who did not lack thiamine and pyridoxine, as they received large amounts of these vitamins i.m., were apparently deficient only in nicotinic acid. They exhibited most of the clinical features of the pure nicotinic acid deficiency syndrome described by Jolliffe *et al.* (1940) and stressed by Lishman (1981). As their histopathological examination was similar to that of the other patients, alcoholic pellagrous encephalopathy and 'pure nicotinic acid deficiency syndrome' are probably identical diseases.

These patients should have responded favourably to niacin therapy, according to Jolliffe *et al.* (1940). However, the response of alcoholic pellagra encephalopathy to nicotinic acid is controversial. According to Still (1976), the resolution of pellagra encephalomyeloneuropathy with nicotinic acid closely parallels that of thiamine deficiency, that is, Wernicke-Korsakoff disease. Nevertheless, Zieve and Hill (1963) have described several patients who developed severe and progressive manifestations of pellagra despite the liberal administration of niacin, other vitamins and a high calorie diet. It may be added that cutaneous manifestations of Case 17 of Spivak and Jackson (1977) resolved after niacin therapy, but the dementia persisted for a prolonged period. Serdaru *et al.* (1981) reported the case of a typical 'late-onset' pellagra encephalopathy in an alcoholic patient who deteriorated on thiamine and pyridoxine therapy and recovered 15 days after the beginning of parenteral nicotinamide therapy. Multiple vitamin therapy should therefore always be adopted for the treatment of undiagnosed encephalopathies in alcoholic patients.

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CHROMATOLYSIS IN ALCOHOLIC ENCEPHALOPATHIES

PELLAGRA-LIKE CHANGES IN 22 CASES

by JEAN-JACQUES HAUW,[1] CÉCILE DE BAECQUE,[1]
CHANTAL HAUSSER-HAUW[1] *and* MICHEL SERDARU[2]

(*From the* [1]*Laboratoire de Neuropathologie Raymond Escourolle and the* [2]*Clinique de Neurologie et de Neuropsychologie, Hôpital de la Salpêtrière, Université Paris VI, Paris, France*)

SUMMARY

In 22 patients with alcoholic encephalopathies, chromatolysis similar to that reported in endemic pellagra was found on postmortem examination. No gross macroscopic changes were seen in affected areas and only neurons were involved. The changes consisted of central chromatolysis, seen predominantly in the brainstem, especially in the pontine nuclei, where they were constant, and in the cerebellar dentate nuclei. Nuclei of cranial nerves (mainly the third, sixth, seventh and eighth), the reticular nuclei, arcuate nuclei and posterior horn cells, were also markedly affected. Changes were sometimes seen in the cerebral cortex, the interpeduncular nuclei, the central mesencephalic grey matter, the colliculi, the tenth and twelfth cranial nerve and perihypoglossal nuclei, the gracile and cuneate nuclei and anterior horn cells. This distribution was different from that reported in endemic and 'endogeneous' pellagra or in isoniazid-induced pellagra encephalopathy. Central chromatolysis was the only pertinent finding of the CNS examination in 9 cases. In 8 cases, chromatolysis was associated with Marchiafava-Bignami encephalopathy, in 4 cases with Wernicke-Korsakoff encephalopathy, and in 1 other case with both. Mild degeneration of spinal cord tracts was seen in 3 cases. The chromatolysis of alcoholic pellagra did not appear to be a retrograde change related to axonal degeneration. Systemic examination showed liver changes in 15/16 cases. Treatment of these cases had not included niacin. No differences were found between cases given thiamine and pyridoxine and those which had not. Microscopic examination of the pons is essential in alcoholic encephalopathies.

INTRODUCTION

The pathological features of 'endemic' pellagra have been well known since the descriptions of Bouchard (1864), Marinesco (1899), Wilson (1914) and Winkelman (1926). Diffuse central chromatolysis is the main change. Similar features can be found in heavy alcohol drinkers with a diagnosis of alcoholic encephalopathy, and such cases have been considered to represent alcoholic pellagra (Meyer, 1963; Ishii and Nishihara, 1981; Duchen and Jacobs, 1984). Descriptions of chromatolysis

Correspondence to: Dr Jean-Jacques Hauw, Laboratoire de Neuropathologie Raymond Escourolle, Hôpital de la Salpêtrière, 47 Bld de l'Hôpital, 75651 Paris Cedex 13, France

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in alcoholic encephalopathies are numerous (Faure, 1899; Turner, 1899; Meyer, 1901; Klauder and Winkelman, 1928; Zimmerman *et al.*, 1934; Schwob *et al.*, 1953; Haguenau *et al.*, 1955; Gruner, 1956; Delay *et al.*, 1958; Deparis *et al.*, 1961; Castaigne *et al.*, 1971; Okeda and Nakano, 1976; Victor and Banker, 1978; Ishii and Nishihara, 1981; Serdaru *et al.*, 1981, 1982; Duchen and Jacobs, 1984; and others). However, they have not led to such a wide recognition of this disorder as that of Wernicke-Korsakoff disease or Marchiafava-Bignami disease. From 1952 to 1983 in the Raymond Escourolle Laboratory, we found diffuse chromatolysis in 22 cases with a clinical diagnosis of alcoholic encephalopathy, selected from 8200 neurological postmortem examinations performed in the same period during which time 111 cases of Wernicke-Korsakoff and 17 cases of Marchiafava-Bignami disease were seen in alcoholic patients. The neuropathological features of these 22 cases are reported here. Their clinical features are given in the preceding paper (Serdaru *et al.*, 1988).

PATIENTS AND METHODS

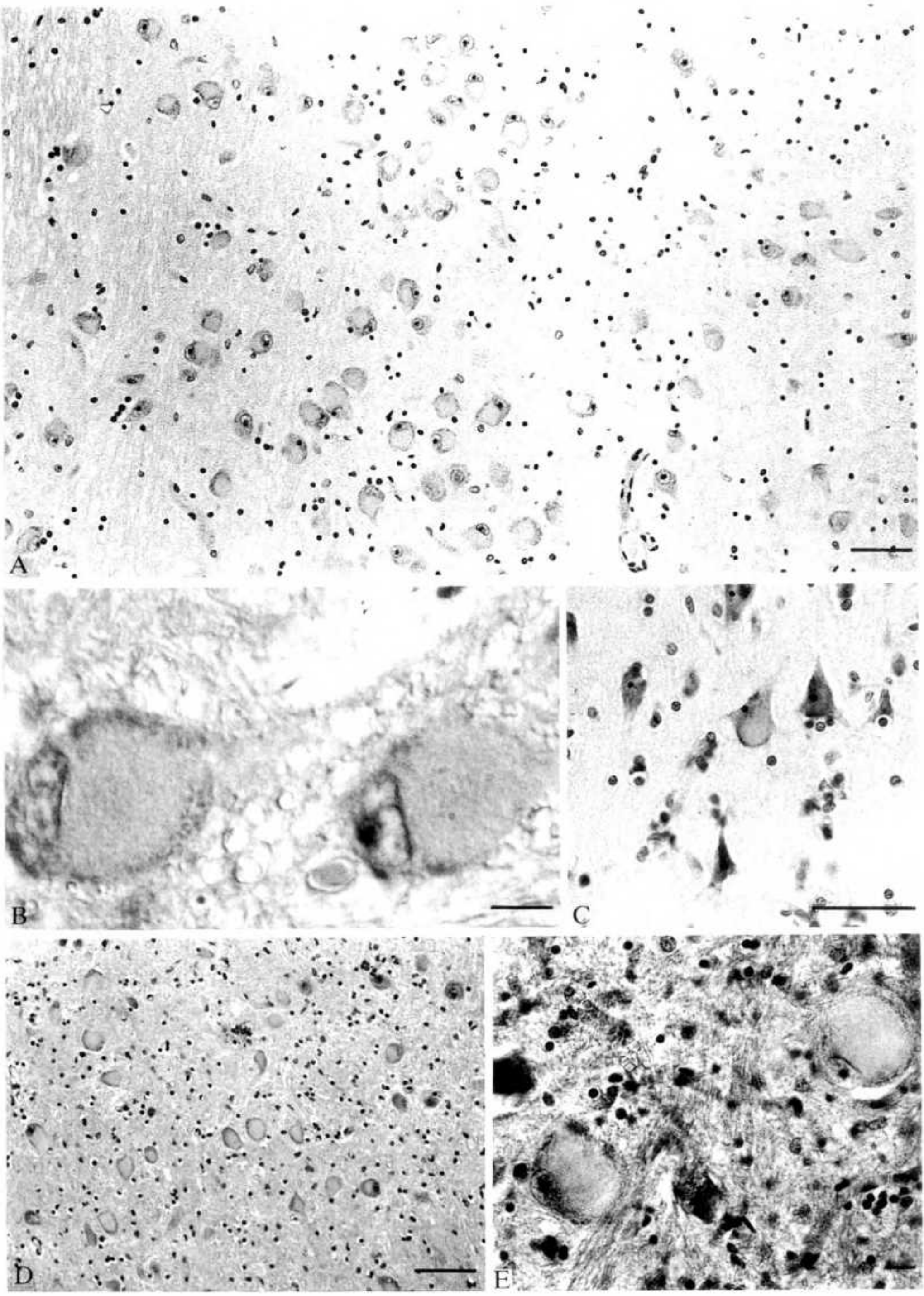
Most patients had been admitted to the Neurology Departments of the Hôpital de la Salpêtrière. Complete postmortem examination was performed in the Raymond Escourolle Laboratory. Only the nervous system (brain and spinal cord or brain alone) of 8 cases referred from other hospitals was examined in this laboratory. Nine of these cases have been reported elsewhere (*see* Serdaru *et al.*, 1988). The brain and spinal cord were fixed in 10% neutral formalin. Coronal sections of the cerebral hemispheres and transverse sections of the brainstem, cerebellum and spinal cord were examined macroscopically and microscopically. The latter study included, in every case, the cerebral hemispheres, at least 3 transverse sections of the brainstem to include the cerebral peduncles, pons and medulla oblongata, and 1 section through the cerebellar vermis and hemispheres, including the dentate nuclei. In 11 cases, one or more sections through the spinal cord were prepared, as indicated in the Table. Samples were embedded in celloidin or paraffin wax and stained with haematein and eosin and, in most cases, by the Nissl technique (thionin), Loyez haematoxylin for myelin and Bodian silver impregnation, depending on the embedding method. For the brainstem nuclei, the nomenclature of Olszewski and Baxter (1982) was used. In 16 cases, tissue sections from different organs, including the liver, were processed for histological examination.

RESULTS

Nervous system

On the basis of the pathological findings, two groups were defined: (1) isolated neuronal chromatolysis, consisting of 9 cases; (2) neuronal chromatolysis with associated alcoholic encephalopathies, comprising 13 cases.

FIG. 1. (*Case 6*). A, nuclei pontis, showing marked and diffuse central chromatolysis. Celloidin, haematein-eosin. Bar = 100 μm B, higher magnification of nuclei pontis, showing eccentric location of nucleus and disappearance of Nissl bodies. Celloidin, haematein-eosin. Nomarski optics Bar = 10 μm. C, frontal cortex. A few neurons show central chromatolysis in the third layer. Celloidin, haematein-eosin. Bar = 100 μm. D, dentate nucleus showing marked and diffuse central chromatolysis. Celloidin, haematein-eosin. Bar = 100 μm. E, nucleus of sixth cranial nerve. Two neurons show obvious chromatolysis. Celloidin, Loyez haematoxylin. Bar = 10 μm.


A
B
C
D
E

Group 1. Isolated neuronal chromatolysis

Neuropathological examination of Cases 1 to 9 showed no changes other than those which could be related to pellagra. Brain weight was 1050–1300 g. Gross examination revealed no marked cerebral or cerebellar atrophy, and the lateral ventricles, corpus callosum, mamillary bodies and brainstem were unremarkable. Microscopic examination did not show any changes suggestive of Wernicke-Korsakoff or Marchiafava-Bignami disease, hepatic encephalopathy and/or cerebellar atrophy.

Nature of the changes. By light microscopy, all cases showed the same changes in the neurons with no associated pertinent changes in glial cells, myelin, blood vessels or meninges. The alterations consisted of ballooning and enlargement with disappearance of the Nissl bodies. Nuclei, eccentrically located, often appeared smaller than normal. These changes constituted the characteristic picture of central chromatolysis, identical to that seen in retrograde degeneration. In a given cell, they were more or less marked. In a given structure, they affected either most neurons or only a few of them. The cytoarchitecture of the central nervous system was otherwise normal.

Topography of the changes. The distribution of the changes was roughly similar in all cases, although the severity of the lesions varied from case to case. They were always bilateral and symmetric. Chromatolysis was more marked within the brainstem, particularly within the pontine nuclei and the cerebellar dentate nuclei.

The pons displayed constant and heavy chromatolysis, affecting the pontine nuclei (figs 1, 2). Large or medium-sized cells from medial, lateral, dorsal and ventral nuclear masses were equally affected. In a few cases, the rostral half was more affected than the caudal. Changes were frequently found in the sixth and seventh nerve nuclei (fig. 1), vestibular nuclei and reticular nuclei, including the nuclei pontis centralis, tegmenti pedunculopontinum, gigantocellularis and parvocellularis. The locus coeruleus, the fifth nerve nuclei, the nuclei of the trapezoid bodies and the inferior collicular nuclei displayed occasional mild involvement. Other neurons were hardly, if ever, affected. The medulla showed prominent chromatolysis in the arcuate and reticulate nuclei (nuclei raphe obscurus and pallidus, nuclei medullae oblongatae centralis, nuclei gigantocellularis, paragigantocellularis, parvocellularis). The hypoglossal and perihypoglossal nuclei, the cuneate, gracile, vestibular, and vagal nuclei were less frequently and less markedly involved. The olivary nuclei, and the nuclei of the tractus solitarius and funiculus lateralis were slightly if at all affected. The mesencephalon exhibited prominent chromatolysis in the third nerve nuclei, the nuclei interpeduncularis and reticularis (nuclei centralis superior, tegmenti pedunculopontinum, griseum centrali mesencephali). The fourth nerve nuclei were less involved. The substantia nigra, the superior colliculi and the corpus Luysii were only slightly affected or not at all. The cerebellum showed very frequent and heavy involvement of the dentate nuclei (fig. 1) and other deep nuclei, contrasting with the sparing of other neurons, including Purkinje cells. The hypothalamus was seldom and then only

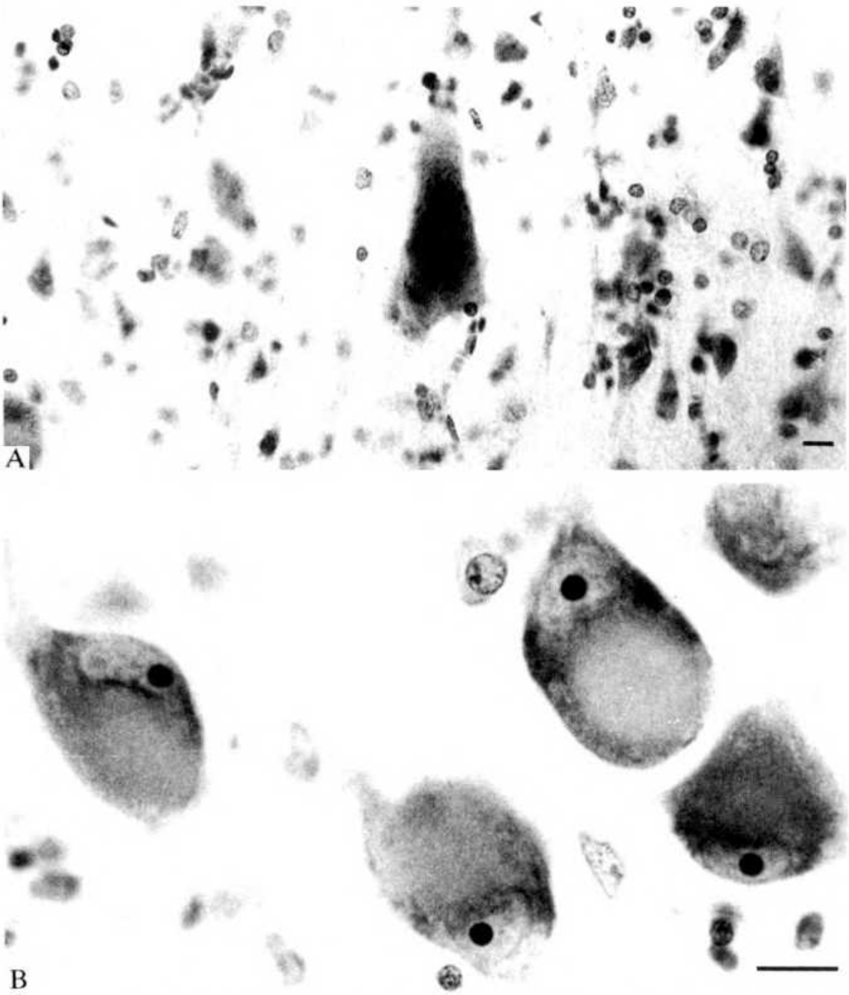


FIG. 2. (*Case 17*). The changes are more marked in the brainstem than in the cerebral cortex. A, precentral area. Note absence of chromatolysis of this Betz cell. Celloidin, Nissl. B, pontine nuclei. Each neuron shows nuclear eccentricity and loss of Nissl bodies characteristic of central chromatolysis. Celloidin, Nissl. Bars = 10 μm.

mildly involved (supraoptic and paraventricular nuclei). The diencephalon and basal ganglia were mostly spared: the mamillary bodies, thalamic nuclei (dorsomedialis and ventralis lateralis) were thoroughly examined and found lesion-free in every case, although scattered chromatolytic neurons could occasionally be seen. The caudate nucleus, putamen and internal and external globus pallidus displayed only very mild or no significant chromatolysis, as did the basal nucleus of Meynert, the nucleus of the diagonal band and the septal nuclei. The cerebral cortex, including the cingulate gyrus and hippocampus (in particular, the Sommer sector), was not involved, with the exception of a few scattered chromatolytic changes in the pyramidal cells of the third and fifth layers, predominantly in the prefrontal

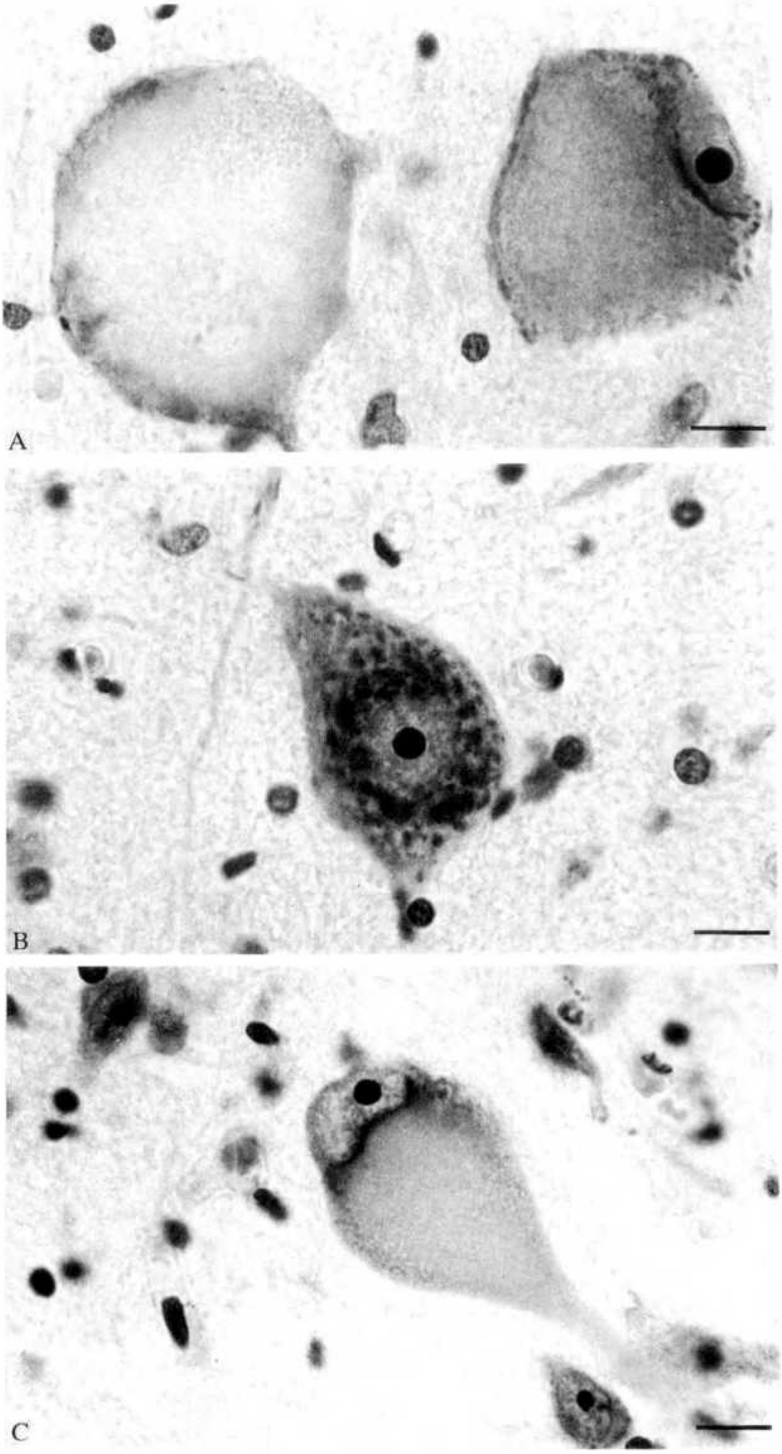


FIG. 3. (*Case 17*). Spinal cord changes. A, central chromatolysis of an anterior horn neuron (anterolateral nucleus) at cervical level. B, normal anterior horn neuron (anterolateral nucleus) at lumbar level. C, chromatolysis of a dorsal horn neuron (nucleus proprius) at thoracic level. Celloidin, Nissl. Bars = 10 μm.

and frontal areas, especially in Betz cells (figs 1, 2). The changes were more prominent in the isocortex in 1 case (Case 3) and the hippocampus in 2 cases (1, 4). The changes in the spinal cord will be described later (fig. 3, Table).

Group 2. Neuronal chromatolysis with associated alcoholic encephalopathies

Cases 10–17 and 22, which exhibited chromatolysis together with lesions of the corpus callosum, were included under the heading *pellagra and Marchiafava-Bignami disease*. The mamillary bodies and thalami were not involved, except in Case 11, where mild chromatolysis was seen in the thalami. Chromatolysis was roughly similar to that of the first group. In Case 15, it was characteristic in the pontine nuclei but mild in the other nuclei. The same pattern was observed in Case 10, where only slight associated involvement of the dentate nuclei was seen. In 5 cases (nos. 10, 11, 14, 17, 22), the corpus callosum displayed thinning and demyelination. In 4 cases (12, 13, 15, 16) necrosis of corpus callosum was seen (recent in Case 16). These changes involved the whole corpus callosum in 5 cases (13, 14, 15, 16, 17), in 1 case (11) the middle third, and in 3 cases (10, 12, 22) the rostral part. In the areas of the heaviest involvement, demyelination or necrosis extended from the corpus callosum, into the centrum semiovale, especially in the cingulate gyrus. In 3 cases (13, 16, 17), the anterior commissure and the optic tracts were also involved. Some neuronal depopulation in the cerebral cortex, which could in some instances be classified as laminar sclerosis, was found in 5 cases (10, 12, 14, 16, 17).

Cases 18–22 displayed chromatolysis and changes of the mamillary bodies indicative of Wernicke-Korsakoff encephalopathy. These cases were included under the heading *pellagra and Wernicke-Korsakoff disease*. In Case 22, fibrillary gliosis of the corpus callosum was also present. In Cases 18 and 22, mamillary changes indicated a longstanding disease (atrophy and gliosis), whereas in Cases 19, 20 and 21 they were recent (haemorrhages, capillary proliferation, status spongiosus and gliosis). In Case 19, changes in the mamillary bodies were associated with milder lesions in the thalamus, mainly in the region of the parafascicular nuclei and the anterior thalamus.

In addition, in Cases 12 and 13 Alzheimer type II astrocytes similar to those seen in hepatic encephalopathy were present in the basal ganglia. No vacuolation of the deep layers of the cortex or of subcortical white matter was seen. In Case 14 there was definite cerebellar atrophy with prominent Purkinje cell loss in the vermis.

In general, the chromatolytic changes associated with Marchiafava-Bignami or Wernicke-Korsakoff disease, especially the latter, were milder. In Cases 18 and 19, they were prominent only in the pontine and vestibular nuclei.

Spinal cord changes. These were assessed in 11 cases from both Groups 1 and 2. The spinal cord was not available for Cases 1, 2, 4, 5, 7, 8, 10, 11, 14, 15 and 22.

TABLE. TOPOGRAPHY AND INTENSITY OF CHROMATOLYSIS IN THE SPINAL CORD

	Case																																
	3			*6**			*9*			*12*			*13*			*16*			*17***			*18***			*19*			*20*			*21*		
	(4474)			*(6271)*			*(2122)*			*(4140)*			*(4125)*			*(3319)*			*(E 517)*			*(4566)*			*(3289)*			*(6305)*			*(6654)*		
	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L	C	T	L
Anterior horn	±	?	±	+	?	++	+	+	+	±	?	+	—	?	++	±	?	?	+++	±	+	—	+	+	+	—	—	—	—	+++	—	±	+
Intermediate grey matter	±	?	—	+	?	—	—	—	—	—	?	—	—	?	—	—	?	?	+	—	+	—	+++	—	+	—	—	—	±	—	—	+	±
Posterior horn	++	?	++	++	?	++	—	+	—	+++	?	++	+	?	+	—	?	?	+	±	+++	+++	—	++	+	—	+	±	—	±	—	++	++

C = cervical; T = thoracic, L = lumbar +++ = severe changes in many or all neurons; ++ = severe changes in some neurons, + = definite changes in few neurons, ± = slight or doubtful changes; — = normal; ? = not available; * = mild degeneration of fasciculus gracilis at cervical level; ** = mild degeneration of fasciculus gracilis at thoracic level.

Although sometimes mild, they were constant (*see* Table). The topography and intensity of chromatolysis were quite variable from one case to another, from one level in the spinal cord to another in the same case and, sometimes, from one side—or even one group of neurons—to the other, at the same level. It was more marked in the posterior horns (nucleus proprius and dorsalis, Clarke's column and, less frequently, the substantia gelatinosa) than in the anterior horns (anteromedial and anterolateral groups). It was milder in the intermediate grey matter. It was more frequent at lumbar than at cervical levels, and was rare in the thoracic region. Mild degeneration of the fasciculus gracilis in 3 cases (16, 17, 18) was the only change seen in the spinal tracts and roots. No degeneration of spinal long tracts was found in Group 1 cases ('pure pellagra').

Systemic examination

With the exception of terminal bronchopneumonia, the only pertinent findings were in the liver, which was examined in 16 cases. In 11 cases, there was isolated alcoholic steatosis, marked in 5 cases (3, 12, 13, 15, 16) and mild in 6 cases (4, 5, 14, 18, 19, 21). In 1 case (19), it was associated with slight mononuclear cell infiltrates in the portal space and mild perilobular fibrosis. In 4 cases (6, 8, 20, 22), early cirrhosis was seen.

DISCUSSION

All the pellagra-like chromatolytic changes seen in this laboratory since 1952 were found to be associated with alcoholism—except for 1 case of endogenous pellagra (Goulon *et al.*, 1969) and for 2 cases associated with Parkinson's disease in elderly patients (J.-J. Hauw *et al.*, unpublished).

The few detailed reports on chromatolysis found in alcoholics account for the controversies that led to the recognition of alcoholic pellagra. The early pathological descriptions of 'axonal reaction' in the cerebral cortex in encephalopathies are due to Faure (1899) in 6 cases and Turner (1899) in 7 cases. Some of these had been alcoholic patients. Meyer (1901) described extensively the diffuse chromatolysis in the cortex, the medulla and in the cerebellar deep nuclei in 2 alcoholic patients. None of these authors mentioned the similarity of these changes to those of pellagra. Klauder and Winkelman (1928) stated that, in a routine examination of more than 2000 cases, the 'so-called central neuritis' (chromatolysis) had been a uniform observation in 2 conditions, namely pellagra (11 cases) and alcoholism, 'especially during the acute stage of delirium tremens'. They did not specify the number of the latter cases. 'Retrograde cell changes' were seen in the spinal cord and in the cortex, and the intimate association between alcoholism and pellagra was stressed. In these early reports, it is often difficult to distinguish alcoholic pellagra from endemic pellagra in alcoholics. Detailed pathological observations of chromatolysis in association with chronic alcoholism have been reported by Zimmerman *et al.* (1934) in 3 cases and Gruner (1956) in 13 of 17, 2 of which had been already reported by Schwob *et al.* (1953). Wernicke-Korsakoff disease was seen in 15, and Marchiafava-Bignami disease in 10. Delay *et al.* (1958) found chromatolysis in each of 8 cases, Wernicke-Korsakoff disease in 7 (1 of them with Marchiafava-Bignami disease). Ishii and Nishihara (1981) reported chromatolysis in 20 cases. Concerning the recognition of chromatolysis, 3 potential errors should be avoided. (1) The presence of eccentrically located nuclei in some structures of the brainstem and spinal cord is normal: in the nucleus of the mesencephalic root of the fifth nerve, the oculomotor and abducens nuclei and the lateral cuneate nuclei (Olszewski and Baxter, 1982) and Clarke's column, for example. However, in some of these nuclei, such as the oculomotor or abducens nuclei, these features are rare and pathological chromatolysis may be ascertained in heavily affected nuclei. (2) Lipofuscin storage, marked, for example, in the dentate nuclei or medullary olives, has to be distinguished from chromatolysis. This can be confusing in elderly patients, especially with haematein-eosin stain. (3) Conversely, ischaemic neuronal changes can mask chromatolysis, and the search for nonischaemic neurons is mandatory for diagnosis.

Chromatolysis could be the result either of retrograde changes following degeneration of cell processes or of a primary cytoplasmic lesion. This study does not favour the first possibility; the cell processes were normal on morphological assessment. Quite severe chromatolysis could be associated with mild changes or normality of the tracts of the spinal cord, as already seen in endogenous pellagra (Goulon *et al.*, 1969).

As far as the distribution of chromatolysis is concerned, the earliest authors, after Faure (1899) and Turner (1899), stressed the changes in the cerebral cortex. However, Meyer (1901) had pointed out the association of chromatolysis of some medullary and cerebellar nuclei with the cortical changes in alcoholic patients. Klauder and Winkelman (1928) mentioned the spinal cord location, Zimmerman *et al.* (1934) the chromatolysis of pontine nuclei. This was confirmed later by most authors although, as late as 1978, Victor and Banker did not mention chromatolysis in pontine nuclei.

Our results are close to those of Koeppen and Barron (1978) and of Ishii and Nishihara (1981). However, we did not find the 'prominent central chromatolysis' of 'nearly all Betz cells' that the former authors described in their case, and the 'constant chromatolytic change in Betz cells' and the very frequent involvement of relatively large cortical neurons in 18 of the 20 cases of the latter authors. In the present study, scarce and mild changes were found in 16 cases. In 6 out of the 22 cases that we studied, the cerebral cortex was indeed considered to be normal. Gruner (1956) and Deparis *et al.* (1961) have stated that cortical neurons were less frequently and less heavily involved than the brainstem nuclei. Some of these differences could be due to difficulties in recognition of this change, as discussed above, or to sampling variations. However, even the examination of numerous sections of the whole hemispheres failed to reveal, in some cases, marked chromatolysis.

The topography of chromatolysis in the brainstem that we observed is close to that described by Gruner (1956), Deparis *et al.* (1961), Koeppen and Barron (1978) and Ishii and Nishihara (1981). The last-named authors found that pontine, gracile and cuneate nuclei were always affected. Chromatolysis was also very frequent in dorsal vagal nuclei (19/20), descending trigeminal nuclei (19/20), vestibular nuclei (19/20), parahypoglossal nuclei (17/20) and, less often, in arcuate nuclei (12/18), dentate nuclei (10/14), ambiguus nuclei (10/20), and oculomotor nuclei (8/16). Changes in some brainstem nuclei were seen in all 22 cases of our series. The pontine nuclei displayed the most severe lesions. These were constant and usually marked in the pontine nuclei and the reticular nuclei of the medulla, pons and mesencephalon. Pigmented nuclei were seldom affected. However, neurons of the nucleus locus coeruleus were sometimes clearly chromatolytic, and this change might have been overlooked in other pigmented cells.

Concerning spinal cord involvement, as far back as the report of Meyer (1901), attention was paid to chromatolysis of spinal cord neurons. In his Case 5, 'very few motor cells', and the cells of Clarke's column 'show the axonal alteration'. The significance of the changes of the neurons of Clarke's column was later considered to merit cautious interpretation because of their normal chromatolysis-like appearance (Wilson, 1914). Klauder and Winkelman (1928) pointed out the involvement of anterior horn cells of the spinal cord in 11 cases, Zimmerman *et al.* (1934) in 2 cases, Victor and Banker (1978) and Ishii and Nishihara (1981) in 3/4 cases. In the present cases, involvement of posterior horn cells was marked and frequent. Schwob *et al.* (1953) in 1 case, and Gruner (1956), have been the only authors to stress the frequent and marked involvement of the cells of posterior horns, in addition to those of Clarke's column—which differed from the normal chromatolysis-like appearance of this nucleus. These changes of posterior horns were associated with milder changes in the anterior horns in the case of Schwob *et al.* (1953).

Chromatolysis was considered to be the only pertinent finding in our series. None of the other changes, such as 'increased lipoid content', 'thickening and hyalinization of the walls of the small vessels' described in the earliest accounts by Klauder and Winkelman (1928) in alcoholic pellagra, and Hsü (1942) in endemic pellagra, were considered to be significant. The changes seen in the 22 alcoholic patients of this series are close to those described in endemic pellagra since the first report by Bouchard (1864), and also in isoniazid-induced (Ishii and Nishihara, 1985) and 'endogeneous' pellagra (Goulon *et al.*, 1969). Two differences may be stressed. (1) The location of chromatolysis was peculiar. In endemic, isoniazid-induced and 'endogeneous' pellagras, in addition to the brainstem nuclei, cortical neurons, especially Betz cells, are always heavily involved (Marinesco, 1899; Greenfield and Holmes, 1939; Hsü, 1942; Zimmerman, 1943; Leigh, 1952; Meyer, 1963; Goulon *et al.*, 1969; Loken, 1971; Smith, 1976; Duchen and Jacobs, 1984; Ishii and Nishihara, 1985; Schochet, 1985). Leigh (1952) states that 'a retrograde cell degeneration of the Betz cells is the only constant pathological finding' and that 'the cells of the pontine, dorsal vagal, gracile and cuneate nuclei show a similar change, but are less constantly affected' in endemic pellagra. It could be hypothesized that the different distribution of chromatolysis in endemic and in alcoholic pellagra is now more obvious with the disappearance of endemic pellagra in developed countries which permits the collection of more numerous examples of 'pure' alcoholic pellagra and to distinguish them from endemic pellagra associated with (or triggered by) alcoholism. In 8 cases of pellagra encephalopathy in tuberculous patients, the distribution of chromatolysis was similar to that of endemic pellagra (Ishii and Nishihara, 1985). In the only pathological report of 'endogenous pellagra', Goulon *et al.* (1969) also observed marked chromatolysis of cerebral cortical neurons and of cranial nerve brainstem nuclei (hypoglossal, vagal, vestibular, cuneate, fifth, fourth, third tegmental reticular nuclei and, especially, pontine nuclei). Dentate nuclei, anterior horns and Clarke's columns were also affected. (2) In endemic pellagra, following Bouchard (1864), degeneration of the posterior and lateral tracts of the spinal cord has been reported by Wilson (1914) in all of 13 cases, Greenfield and Holmes (1939) in 1 case, Hsü (1942) in 11 of 13 cases and Leigh (1952) in 3 of 6 cases. In the 22 cases of this series, only 3 instances of mild degeneration of the fasciculus gracilis were found. Degeneration of the gracile and cuneate fasciculi, and of lateral and anterior pyramidal tracts have nevertheless been described in 2 cases of alcoholic pellagra by Zimmerman *et al.* (1934). Conversely, more recent reports of Schwob *et al.* (1953) in Case 1, and Ishii and Nishihara (1981) in 2 cases did not find degeneration of spinal cord tracts. In this series, it must be emphasized that no degeneration of long tracts was found in group 1 ('pure pellagra').

Examination of the liver, available in 16 cases, differed from the marked hepatic disorders which are usually associated with chronic hepatic encephalopathy (Victor *et al.*, 1965). However, in 2 cases (12, 13), neuropathological examination disclosed Alzheimer type II astrocytes which are usually encountered in hepatic encephalopathy (Victor *et al.*, 1965). Marked fatty change in the liver was observed in these 2 cases but blood levels of ammonia were unknown. They were classified as hepatic encephalopathies associated with pellagra.

We would like to stress the frequent association of changes indicative of other alcoholic encephalopathies in this series. Isolated chromatolysis was found only in 9 of 22 cases. Diffuse chromatolysis has previously been mentioned in pathological reports of various alcoholic encephalopathies, especially Wernicke-Korsakoff disease (Schwob *et al.*, 1953), Marchiafava-Bignami disease (Castaigne *et al.*, 1971) or both (Gruner, 1956; Delay *et al.*, 1958; Koeppen and Barron, 1978). Several authors described chromatolysis in Wernicke-Korsakoff disease without specifically mentioning pellagra (Carmichael and Stern, 1931; Schwob *et al.*, 1953; Gruner, 1956; Delay *et al.*, 1958; Koeppen and Barron, 1978). However, Torvik *et al.* (1982) and Harper (1983) did not mention central chromatolysis in any cases of Wernicke-Korsakoff disease. In this laboratory, the association of alcoholic pellagra and Marchiafava-Bignami disease is more frequent

(9/17 cases of this disease) than that of alcoholic pellagra and Wernicke-Korsakoff disease (5/111 cases of this disease). This difference is statistically significant ($\chi^2 = 35.5$, $P < 0.00001$). This may indicate some common and as yet unrecognized mechanism. Among the 22 reported patients, 9 developed pellagra after their admission to the hospital under daily high-dose parenteral thiamine and pyridoxine therapy (Serdaru *et al.*, 1988). The pathological changes did not differ from those of untreated cases of alcoholic pellagra (Serdaru *et al.*, 1981). On practical grounds, we would like to emphasize the need for microscopic examination of the brainstem in alcoholic patients.

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CLINICAL AND MORPHOMETRIC ANALYSIS OF BIOPSIED BICEPS BRACHII MUSCLES IN ADULT-ONSET CHRONIC PROXIMAL SPINAL MUSCULAR ATROPHY

WITH SPECIAL REFERENCE TO INTRAMUSCULAR NERVES

by R. L. ROSALES,[1,2,3] M. OSAME,[1,2] M. KOUKA,[1] K. ARIMURA,[2]
H. NAKASHIMA[1] *and* J. C. NAVARRO[3]

(From the [1]Muscle, Nerve and [2]Electrophysiology Laboratories of the Third Department of Internal Medicine, Faculty of Medicine, Kagoshima University, Japan, and the [3]Neuromuscular Unit, Neurology Section, Department of Neurology and Psychiatry, Faculty of Medicine and Surgery, University of Santo Tomas, Manila, Philippines)

SUMMARY

In a group of 16 cases with adult-onset chronic proximal spinal muscular atrophy, we performed a morphometric analysis of 88 intramuscular nerves in biopsied biceps brachii muscles and correlated this analysis with clinical and histochemical parameters. The total number of large and small myelinated fibres in all fascicles was reduced to 71%, 23% and 83% of control values, respectively. The percentage reduction of large myelinated fibres for each case was significantly correlated with duration of illness, biceps muscle power and histochemical atrophy factors. Histograms of large intramuscular nerve fascicles showed unimodal distributions and shifts to the left with single peak increases at 1 μm. The densities of small myelinated fibres in large fascicles correlated with counts of 'enclosed' muscle fibres in ATPase preparations. Compared with amyotrophic lateral sclerosis, a lesser reduction of large myelinated fibres, but a greater increase in small myelinated fibres, was noted in adult-onset chronic proximal spinal muscular atrophy. These findings imply that there is a less marked loss of myelinated nerve fibres with more effective reinnervation in adult-onset chronic proximal spinal muscular atrophy than in amyotrophic lateral sclerosis.

INTRODUCTION

The motor neuron diseases (Rowland, 1982) are clinically heterogeneous and include the spinal muscular atrophies (SMA), also designated as motor neuronopathies (Thomas, 1981), and hereditary motor neuropathies (HMN) (Harding, 1984). To date, extensive and pooled reports (Emery, 1971; Emery *et al.*, 1975, 1976*a*, *b*; Pearn, 1980) have resolved some nosological questions that beset clinicians. Our efforts concern one such group, the adult-onset chronic proximal spinal muscular

Correspondence to: Professor M. Osame, Third Department of Internal Medicine, Faculty of Medicine, Kagoshima University, 1208-1 Usuki-cho, Kagoshima 890, Japan.

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atrophies (Bundey and Lovelace, 1975) (abbreviated to adult-onset SMA in this communication). In many ways, such as late onset, male predominance, scarcity of sensory manifestations, neurogenic changes in both routine electromyography and muscle biopsy, adult-onset SMA resembles, apart from the lack of upper motor neuron involvement, another motor neuron disease, amyotrophic lateral sclerosis (ALS). Indeed, their differentiation employing several parameters has become the subject of some earlier reports (Namba *et al.*, 1970; Mastaglia and Walton, 1971; Ross *et al.*, 1974; Horton *et al.*, 1976; Pearn *et al.*, 1978; Corbett *et al.*, 1982). The need for these specifications relates to their prognostic and genetic implications, and the problem can be compounded if the cases with adult-onset SMA have no clear familial background. However, recent quantitative electrophysiological data (Stålberg *et al.*, 1975; Stålberg, 1982; Swash and Schwartz, 1982; Stålberg and Sanders, 1984; *see also* reviews by Bradley, 1987) have provided excellent insights for their differentiation, especially when coupled with histochemistry of biopsied muscles (Fawcett *et al.*, 1985). In this report, we shall attempt to answer three questions. (1) To what degree and extent are intramuscular nerves involved in adult-onset SMA? (2) Can the quantitated data so obtained be useful for correlations with certain clinical and histochemical features? (3) How does the morphometric analysis of intramuscular nerves in adult-onset SMA compare with ALS? Morphometric data thus acquired will not only help in assessment of pathological mechanisms, but may also provide further clues in differentiating ALS from the infrequently reported cases of adult-onset SMA. To our knowledge, these approaches have not been made in the past.

MATERIAL AND METHODS

Cases

Muscle biopsies from 16 cases (13 men and 3 women) taken from biceps brachii and which contained intramuscular nerves, formed the material for this study. The diagnosis of adult-onset SMA was based on modifications of existing criteria (Bundey and Lovelace, 1975; Pearn *et al.*, 1978). These expanded criteria included (1) onset at 15 yrs or older; (2) insidious evolution of symptoms with a benign course; (3) predominant proximal symmetric muscle weakness; (4) absence of obvious cranial nerve and pyramidal signs, as well as sensory involvement; (5) a neurogenic pattern on routine electromyography (EMG), with normal motor and sensory conduction studies; and (6) exclusion of other neurological disorders by detailed clinical examination, radiological tests and laboratory studies (including serum glucose, cholesterol, triglyceride and lipoprotein assay and CSF analysis). Attempts were likewise made to exclude patients with similar disorders in the family suggesting autosomal dominant or X-linked patterns. No case with gynaecomastia and/or testicular atrophy was included. Table 1 shows that the ages at the time of biopsy ranged from 24 to 63 yrs (median 52 yrs), and the age range at clinical onset was 15 to 55 yrs (median 35 yrs). Only 2 cases had a family history of consanguinous marriage. No case was able to walk unaided after 20 yrs of illness.

Morphological methods

Biopsied biceps muscles in all cases were quenched in isopentane, which had been chilled in liquid nitrogen, and serial transverse sections made in a cryostat. Using standard methods, frozen sections

TABLE 1 CLINICAL DATA OF 16 CASES WITH ADULT-ONSET SMA

			Age (yrs)			
Case	*Age/sex*	*Family history*	*Clinical onset*	*Needed assistance in ambulation*	*Wheelchair-bound*	*EMG*
1	57/M	—	37	48	—	N
2	43/M	—	17	30	—	N
3	61/F	—	28	57	60	N
4	54/F	+	31	46	52	N
5	55/M	+	33	49	—	NM
6	50/M	—	45	—	—	N
7	53/M	—	46	—	—	N
8	43/M	—	35	—	—	N
9	59/M	—	46	—	—	N
10	38/M	—	33	—	—	N
11	24/M	—	15	—	—	N
12	52/M	—	42	—	—	N
13	36/M	—	15	23	—	N
14	63/F	—	55	—	—	N
15	52/M	—	15	34	52	N
16	46/M	—	37	—	—	N

M = male; F = female, + = consanguinous marriage; — = nothing particular/has not occurred, N = neurogenic; NM = predominantly neurogenic with few brief small amplitude potentials.

were stained with haematoxylin-eosin, modified Gomori trichrome, nicotine adenine dinucleotide-tetrazolium reductase, cytochrome c oxidase, adenosine monophosphate deaminase, adenosine triphosphatase (ATPase), acid phosphatase, periodic acid-Schiff, oil red-O and Sudan black B. From among the typical changes of chronic neurogenic atrophy found in these cases (*see* Swash and Schwartz, 1984; Dubowitz, 1985), two were thought to be useful parameters for quantitation. These changes are (1) individual small fibres with concave borders reacting to both histochemical fibre types, and/or those occurring in groups; and (2) 'enclosed fibres' which are completely surrounded by fibres of their own histochemical type. Particular emphasis was thus placed on ATPase stains (pH 4.33, 4 5 and 10.4) for determination of fibre types using the method of Dubowitz (1985). Random frames of 5 were photographed in each section, and fibre diameters of 3 histochemical types (I, IIA and IIB) were measured using a Zeiss TGA 10 particle analyser (final magnification, ×200) This was followed by a programmed calculation for histographic analysis. The numbers of 'enclosed fibres' based on the hexagonal lattice model (Johnson *et al.*, 1973*a*) and calculated atrophy factors adjusted for sex (Dubowitz, 1985) were summed for each fibre type to obtain the total enclosed fibre counts (EFC) and total atrophy factors (AF) for every biopsy specimen.

Portions of the specimens fixed in 3% glutaraldehyde, osmicated and embedded in epon, were cut transversely and stained with toluidine blue and safranine for morphometric analysis of intramuscular nerves (Madriaga *et al.*, 1985; Rosales *et al.*, 1988). After obtaining the diameters, intramuscular nerve fascicles were divided into 6 groups. This was useful for assessing involvement of proximal sites, and therefore large fascicles, and distal sites with small fascicles (Rosales *et al.*, 1988). According to intraperineurial diameters, the mean and SD of the number of myelinated fibres (MF) can be calculated. The following were then determined: (1) the intraperineurial diameter, where the shortest intraperineurial length was used, when the fascicles were oval in shape; (2) the number of MFs per fascicle; and (3) fibre size distribution and diameter spectra of MF/mm^2 of intraperineurial area. Quantitative data so obtained were correlated with duration of illness, clinically determined biceps muscle power (MRC grading, 1982), histochemical AF and EFC. Comparison was likewise made with our recently reported morphometric data of intramuscular nerves in ALS (Rosales *et al.*, 1988). In all, we specifically chose the biopsied biceps brachii for adequate reference to our published normative data on 5 autopsy cases with age range of 33–63 yrs and median of 40 yrs (Madriaga *et al.*, 1985). These control data were further expanded by adding 10 other biceps brachii biopsy specimens, for which neither electrophysiological nor histological studies revealed abnormalities. The biopsied subjects consisted of 6 males and 4 females, whose ages ranged from 24 to 62 yrs, and with a median of 50 yrs. They were primarily referred to in order to exclude metabolic muscle disorders. Most of them complained of what were described as 'muscle cramps'. Extensive laboratory investigations, including ischaemic forearm exercise tests, performed in 5 of the subjects, and the biochemical studies in muscles of all subjects were unremarkable.

For the various comparative analyses, the observer (R.L.R.) made blind assessments. In the slides of the two sets of controls, and those of adult-onset SMA and ALS, all the labels were uniformly covered and then intramuscular nerve fascicles were photographed. After having made diameter measurements, appropriate labels for each group were specified.

RESULTS

Histochemistry

Other than the predominant features of chronic neurogenic atrophy (*see* Swash and Schwartz, 1984; Dubowitz, 1985), and the 8 cases showing mild myopathic changes, which were mostly internal nucleation and fibre splitting, no other remarkable histochemical changes were noted. In only 4 cases were there significant hypertrophy factors (Dubowitz, 1985). The AF and EFC values are listed in Table 2 and a typical muscle histogram is shown in fig. 1. Atrophic fibres were

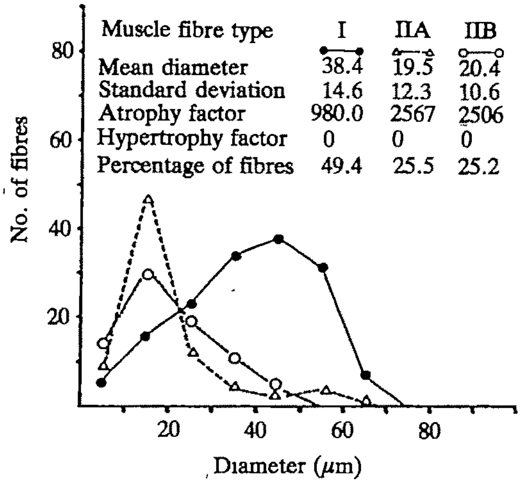

FIG. 1. Representative muscle fibre size distributions from a case of adult-onset SMA. Computation for atrophy factors is taken by multiplying the number of fibres with diameters 30–40 μm by 1, 20–30 μm by 2, 10–20 μm by 3, and the number in the group < 10 μm by 4 These products are added together and divided by the total number of fibres. The result is then multiplied by 1000. Hypertrophy factors are similarly divided for the proportion of fibres in the hypertrophic range.

TABLE 2. DATA ON FROZEN MUSCLE HISTOCHEMISTRY OF 16 CASES WITH ADULT-ONSET SMA

	General	*Type I*			*Type IIA*			*Type IIB*			*Total*	*Total*
Case	*findings*	%	*AF*	*EFC*	%	*AF*	*EFC*	%	*AF*	*EFC*	*AF*	*EFC*
1	NM	49	980	52	26	2587	0	25	2506	0	6073	52
2	N	47	117	34	15	1448	0	38	210	41	1775	75
3	NM	48	273	64	26	76	0	26	173	0	522	64
4	NM	74	27	51	17	68	0	9	156	0	251	51
5	NM	57	948	93	21	2744	0	22	2697	0	6389	93
6	N	63	94	118	20	292	0	17	30	30	416	148
7	N	63	8	70	21	0	18	16	64	13	72	101
8	NM	53	803	16	23	489	0	24	673	0	1965	16
9	NM	55	154	65	23	130	0	22	636	0	920	65
10	N	50	70	13	26	0	0	24	0	0	70	13
11	N	58	176	91	21	111	11	21	22	22	309	124
12	NM	44	1479	73	29	506	0	27	792	0	2777	73
13	NM	60	680	78	20	320	0	20	410	0	1410	78
14	N	48	790	101	27	543	0	25	547	0	1880	101
15	N	46	104	84	27	1887	13	27	976	19	2967	116
16	N	45	270	83	27	2606	19	28	2419	13	5295	115

N = neurogenic atrophy; NM = mainly neurogenic atrophy, with slight myopathy; % = percentage of fibre type; AF = atrophy factor; EFC = enclosed fibre counts.

found in all fibre types. However, in both types IIA and IIB, atrophy factors were significantly higher ($0.1 < P < 0.2$) than type I fibres. The respective percentages of the 3 fibre types showed that there was a wide variation in proportions between the sampled biceps muscles of the cases with adult-onset SMA (Table 2). The number of 'enclosed fibres' noted in the actual samples were compared with the number expected to occur in a hexagonal lattice model (Johnson *et al.*, 1973*a*). A significant increase of 'enclosed fibres' was noted in all the fibre types, but considerably greater ($P < 0.005$) in type I than either type IIA or IIB. The AF correlated inversely with clinically graded biceps muscle power ($r = -0.45$, $0.05 < P < 0.1$).

Pooled data on morphometric analysis of intramuscular nerves in controls

A total of 50 intramuscular nerves was analysed from the 10 biopsied subjects (5/biopsy). In all groups of fascicular diameters (Groups I–VI), the total number and counts of large MF ($\geqslant 5$ μm) and small MF (< 5μm) did not vary statistically from our data on autopsied cases (Madriaga *et al.*, 1985). Similarly, the diameter histograms showed a unimodal fibre size distribution in the small fascicles for Groups II–IV, while a bimodal fibre size distribution with peaks at 2 and 7 μm was noted in the large fascicles for Groups V–VI. These results justified averaging the pooled figures of both autopsied and biopsied subjects, with a total of 15 controls.

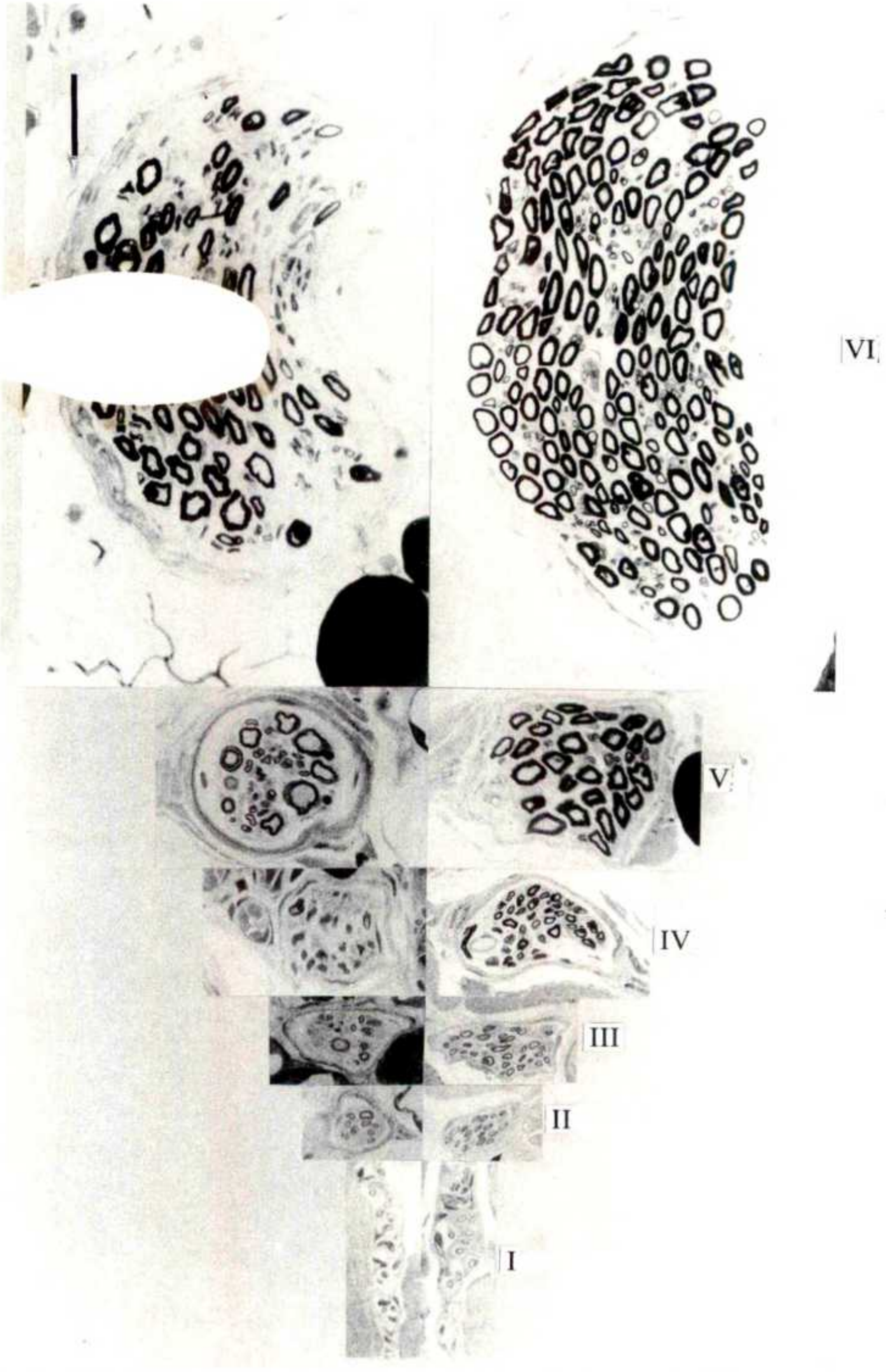

FIG. 2. Representative intramuscular nerve fascicles of controls (*right*) and adult-onset SMA (*left*). To represent various levels are: Group VI, fascicles ≥ 70 μm in diameter; Group V, fascicles 50–70 μm in diameter; Group IV, fascicles 30–50 μm in diameter; Group III, fascicles 20–30 μm in diameter; Group II, fascicles 10–20 μm in diameter; Group I, fascicles 1–10 μm in diameter. Bar = 30 μm. Note the reduction in myelinated fibre numbers at all levels of intramuscular nerve fascicles in adult-onset SMA.

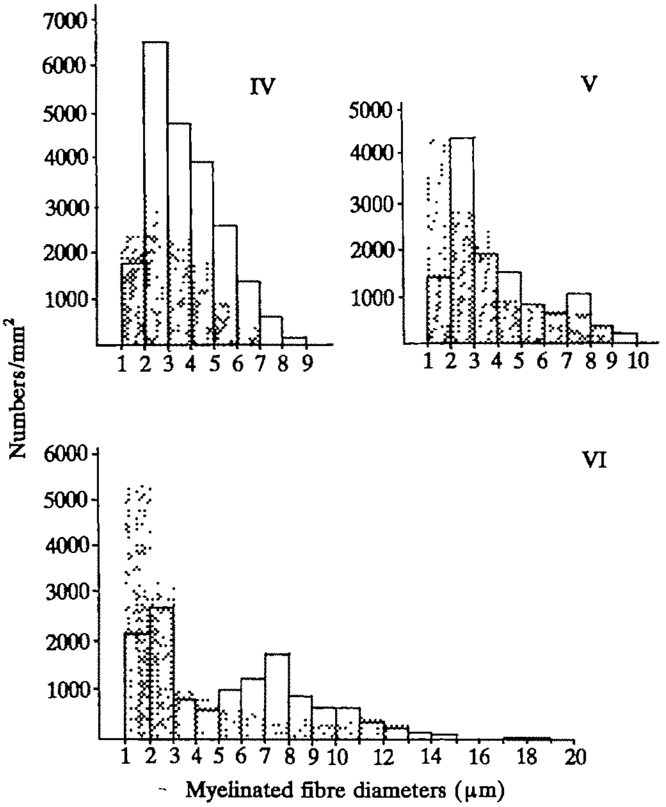

FIG. 3. Diameter histograms for Group IV–VI fascicles in controls (open areas bounded by black lines) and cases of adult-onset SMA (shaded areas). Note the unimodal distributions and the shift to the left in Groups IV–VI. Groups V–VI are shifted to the left with single peak increases at 1 μm.

Pooled data on morphometric analysis of intramuscular nerves in adult-onset SMA

Eighty-eight intramuscular nerves were analysed, and the number of nerve fascicles studied ranged from 3 to 8 per biopsy. Compared with the mean for controls (fig. 2; Table 3) and total MF counts, mainly for the large MFs, were reduced. Counts of small MFs also decreased, but tended to increase in the large fascicles, Groups V (104.5% of controls, $P > 0.2$) and VI (136.3% of controls, $P > 0.2$) (Table 3). For all groups of fascicles combined, the mean reductions in the counts of total, large and small MFs were 71% ($P < 0.005$), 23% ($P < 0.005$) and 83% ($0.005 < P < 0.01$) of controls, respectively. Fibre diameter histograms revealed loss of the density of large MFs and increase in the peak of small MFs in the large fascicles, Groups V and VI (Table 4; fig. 3). The normal bimodal distributions seen in the large fascicles of controls were not retained, unimodal distributions being present, with shifts to the left and single peak increases (1 μm).

TABLE 3. COUNTS OF MYELINATED FIBRES IN THE INTRAMUSCULAR NERVE FASCICLES OF CONTROLS AND ADULT-ONSET SMA

Fascicle groups	*Total no. of MFs (mean ± SD)*	*Large MFs (≥ 5 μm) (mean ± SD)*	*Small MFs (< 5 μm) (mean ± SD)*
I (1- < 10 μm)			
Control	1.13 ± 0.40	0	1.13 ± 0.40
Adult SMA	1.07 ± 0.55	0	1.07 ± 0.55
Percentage*	94.7	0	94.7
II (10- < 20 μm)			
Control	5.29 ± 4.27	0.17 ± 0.14	5.12 ± 4.22
Adult SMA	2.37 ± 1.65	0	2.37 ± 1.65
Percentage*	44.8	0	46.3
III (20- < 30 μm)			
Control	14.00 ± 6.69	0.92 ± 1.06	13.08 ± 6.71
Adult SMA	8.9 ± 4.43	0.3 ± 0.67	8.6 ± 4.06
Percentage*	63.6	32.6	65.7
IV (50- < 70 μm)			
Control	24.61 ± 10.93	5.28 ± 5.19	19.33 ± 8.64
Adult SMA	11.17 ± 3.9	1.08 ± 0.9	10.08 ± 3.03
Percentage*	45.4	20.5	52.1
V (50- < 70 μm)			
Control	32.21 ± 9.40	8.29 ± 4.84	23.93 ± 10.32
Adult SMA	29.33 ± 10.6	4.33 ± 3.79	25 ± 13.53
Percentage*	91.1	52.2	104.5
VI (70- > 70 μm)			
Control	105.08 ± 29.73	51.67 ± 20.50	53.42 ± 13.42
Adult SMA	90.67 ± 32.37	17.83 ± 10.19	72.83 ± 27.92
Percentage*	86.3	34.5	136.3

* Expressed as percentage of adult-onset SMA to controls; Adult SMA = adult onset chronic proximal spinal muscular atrophy; MF = myelinated fibres.

TABLE 4. DIAMETERS AND DENSITIES OF MYELINATED FIBRES/mm^2 AREA OF FASCICLE IN CONTROLS AND ADULT-ONSET CHRONIC PROXIMAL SPINAL MUSCULAR ATROPHY

	Groups of fascicles				
	II	*III*	*IV*	*V*	*VI*
Diameter of MFs (μm)					
Controls	1-5	1-7	1-18	1-9	1-9
Adult-onset SMA	1-4	1-6.5	1-7	1-9	1-12
Total MF density/mm^2 area					
Controls	32,063	28,100	21,185	12,366	12,036
Adult-onset SMA	17,057	19,122	9,871	11,745	12,564
Large MF density/mm^2 area					
Controls	1,011	1,853	4,373	3,369	5,863
Adult-onset SMA	0	435	958	1,869	2,541
Small MF density/mm^2 area					
Controls	31,052	26,247	16,812	8,997	6,173
Adult-onset SMA	17,057	18,687	8,913	9,876	10,023

Note: Group I MF diameters in control and adult-onset SMA (adult-onset chronic proximal spinal muscular atrophy) were 1-6 μm and 1-4 μm, respectively; densities of MF in Group I were not calculated (*see* Madriaga *et al.*, 1985); MF = myelinated fibres.

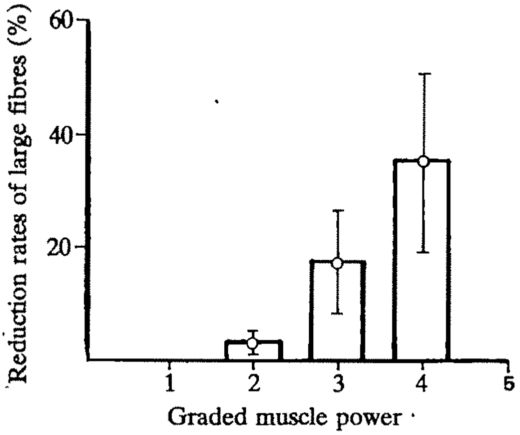

FIG 4. Bar diagram showing the correlation between graded muscle power and reduction rates of large fibres. Peaks indicate mean ± SD. Reduction rates indicate percentage of large MFs in adult-onset SMA compared with controls

Individual analysis of intramuscular nerves and feature correlations

In order to formulate correlations for each case, we obtained the mean percentage reduction of MFs per biopsy in adult-onset SMA as compared with controls. The percentages were expressed as reduction rates of MFs such that the lower the values, the greater is the loss of MFs. Reduction rates of large MFs significantly correlated with clinically graded muscle power in biceps brachii ($r = 0.711$, $P < 0.005$; fig. 4) and AF ($r = -0.46$, $0.05 < P < 0.1$; fig. 5). Although reduction rates of large MFs likewise correlated with illness duration of $\leqslant 20$ yrs ($r = -0.48$, $0.05 < P < 0.1$, fig. 6), longer durations had reduction values < 8% of controls. Since pooled analysis of intramuscular nerves revealed a tendency for the numbers of small MFs to increase in large fascicles of adult-onset SMA, these densities were determined individually per fascicular area for purposes of

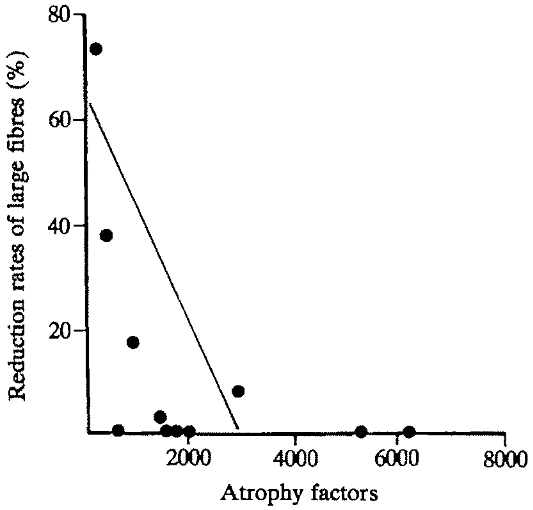

FIG. 5. Scattergram showing regression analysis of the correlation between atrophy factors and reduction rates of large fibres; $\hat{Y}$ = predicted reduction rate for every value of X (atrophy factor) ($-50.54 \times +(2927.19)$; SEE = standard error of the estimate (1979). Reduction rates indicate percentage of large MFs in adult-onset SMA compared with controls.

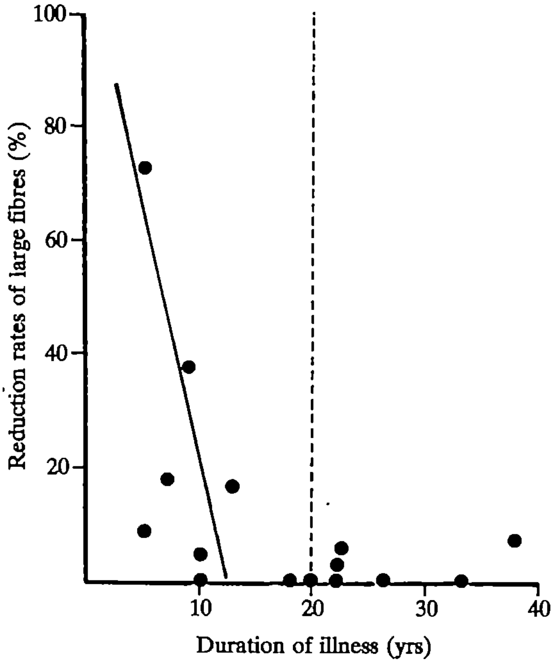

FIG. 6. Scattergram showing regression analysis of the correlation between duration of illness and reduction rates for large fibres. Plotted points can be divided visually, with 20 yrs as the cut-off; for those $\leqslant 20$ yrs, $\hat{Y}$ is the predicted reduction rate for every value of X (illness duration) $(-0.10 \times +(12.07)$, and SEE is the standard error of the estimate (4.68). For those > 20 yrs, reduction rates clustered at $< 8\%$. Reduction rates indicate percentages of large MFs in adult-onset SMA compared with controls.

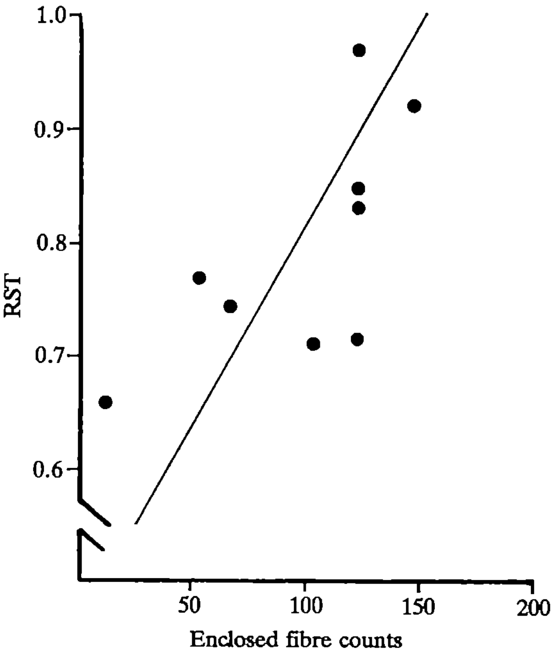

FIG. 7. Scattergram showing regression analysis of the correlation between the density ratio of small MFs and total MFs per fascicular area (RST) and enclosed fibre counts in Groups V and VI; SEE = standard error of the estimate (35.6). $\hat{Y} = 288.57 \times +(-134.26)$.

TABLE 5 COMPARATIVE MORPHOMETRIC ANALYSIS OF INTRAMUSCULAR NERVES IN ALS AND ADULT-ONSET SMA

	ALS	*Adult-onset SMA*
Cases	13 M:3 F	13 M:3 F
No. of intramuscular nerves studied/biopsy	5.6±1.6	5.5±1.5
Percentage vs controls		
Total MF	52%	71%*
Large MF	16%	23%*
Small MF	64%	83%*
RST in large fascicles	0.67±0.17	0.80±0.10*

* Adult-onset SMA significantly higher than ALS, by Student's t test; RST = density ratio of small MF and total MF per fascicular area; ALS = amyotrophic lateral sclerosis; adult-onset SMA = adult-onset chronic proximal spinal muscular atrophy; MF = myelinated fibres. For complete ALS data, *see* Rosales *et al.* (1988)

correlation. The scattered values of individual small MF densities further led us to obtain the ratio of densities between small MFs and the total for more appropriate statistical correlation with EFC. Compared with the controls (range: 0.23–0.78), the ratio in adult-onset SMA (range: 0.67–0.97) was significantly higher ($t = 3.7$, $P < 0.005$) and was also significantly correlated with EFC ($r = 0.66$, $0.05 < P < 0.1$; fig. 7).

Comparative analysis of intramuscular nerves in adult-onset SMA and ALS

The pooled morphometric data of intramuscular nerves in adult-onset SMA were compared with those from our recent report on ALS (Rosales *et al.*, 1988) using the same morphological methods. Although both in ALS and adult-onset SMA, MF numbers decreased at all levels in intramuscular nerve fascicles, significantly greater losses of total counts ($0.1 < P < 0.2$), and large ($0.005 < P < 0.01$) and small ($0.05 < P < 0.1$) MFs were noted in ALS (Table 5). In the large fascicles, both disorders a shift to the left and unimodal distributions, although the single peak increases (1 μm) were obviously higher in adult-onset SMA. The ratio of the small fibre density to total fibre density determined in individual cases of ALS (range: 0.33–0.88) was significantly lower than in adult-onset SMA ($t = 2.05$, $0.05 < P < 0.1$).

DISCUSSION

In cases with adult-onset SMA, segregation analysis by Pearn *et al.* (1978) indicated that the majority are of autosomal recessive inheritance (termed type IV SMA by Emery, 1971, and type III proximal HMN by Harding, 1984). Although the present 16 cases with adult-onset SMA do not show significant family histories, a recessive pattern remains a strong possibility, considering that most of those that appear to be sporadic cases may be the result of recessive inheritance (Emery *et al.*, 1975; Harding, 1984). We assumed, although the possibility is not completely ruled out (Wilde *et al.*, 1987), that X-linked recessive forms were not included, based on the lack of family history and the absence of bulbar signs and gynaecomastia (Kennedy *et al.*, 1968; Tsukagoshi *et al.*, 1970; Stefanis *et al.*, 1975; Ringel *et al.*, 1978; Mukai, 1980; Harding *et al.*, 1982; Arbizu *et al.*, 1983; Wilde *et al.*, 1987). Moreover, the salient clinical features, electrophysiological tests and histochemical findings in biopsied muscles of our patients are compatible with previously reported sporadic and autosomal recessive forms (Meadows *et al.*, 1969; Namba *et al.*, 1970; Pearn *et al.*, 1978; Russman *et al.*, 1983; Carpenter and Karpati, 1984; Swash and Schwartz, 1984).

Few cases of adult-onset SMA have been reported, since they form a difficult entity to define in diagnostic terms (Harding, 1984) in relation to other disorders such as progressive muscular atrophy (ALS) and similar forms of slowly progressive motor neuronopathy. It is precisely because of this that we attempted to expand our diagnostic criteria for adult-onset SMA. Even so, its limitations led us to explore avenues that may provide other clues for differentiation. Our interest focused on muscle nerves of available biopsies where notable changes in motor neuron disorders have previously attracted attention (Carpenter and Karpati, 1984; Swash and Schwartz, 1984). Quantitative intramuscular nerve analysis provides further information on the amount and extent of involvement and can be potentially useful in elucidating pathological mechanisms (Rosales *et al.*, 1988). For instance, we were able to document decreased numbers and sizes of MFs in the intramuscular nerves of the patients with adult-onset SMA studied here. Not only is this method advantageous in these respects, but also in relation to clinical features and quantifiable histochemical parameters. We can thus derive some inferences from our findings. (1) The involvement extends to all levels, from large fascicles (proximal sites) to small fascicles (distal sites) of even the remote part of the nerve to the muscle. (2) The relative increase in numbers of small MFs in large fascicles suggests either atrophy of large MFs or axonal sprouting. Since the diameter spectra displayed a shift to the left and single peak increases in these large fascicles, both conditions probably coexist. (3) The afferent fibres may also be involved, since both the total number and numbers of large MFs were reduced (Rosales *et al.*, 1988). (4) The degree of loss of motor innervation of muscle fibres can be reflected in the reductions of MFs in the intramuscular nerves. (5) The process is dynamic and progressive up to a certain extent (note the almost constant reduction rates for large fibres in cases with illness duration $>$ 20 yrs).

Although in muscle biopsies of motor neuron disorders, findings compatible with neurogenic atrophy are usually found, comparative morphometric intramuscular nerve analysis may yet be another useful tool that can provide further points of differentiation. This is true in the present study comparing adult-onset SMA and ALS. Our findings imply that there is a less marked loss of MFs with more effective reinnervation in adult-onset SMA than in ALS. Thus adult-onset SMA patients have a relatively benign course and a better prognosis, as affected muscles are able to compensate by an increase in motor unit territories. The conclusion that the sprouting process plays an important role in the pathology of adult-onset SMA finds support from the association between our small to total fibre density and EFC and the raised terminal innervation patterns found by Cöers *et al.* (1973). Fibre type grouping, quantitated here as EFC, is established evidence favouring reinnervation (Carpenter and Karpati, 1984; Fawcett *et al.*, 1985). Higher fibre densities to suggest reinnervation in single-fibre EMG have also been reported in SMA as compared with ALS (Stålberg *et al.*, 1975; Stålberg, 1982; Swash and Schwartz, 1982; Stålberg and Sanders, 1984). These differences could arise if in ALS the surviving neurons are compromised and incapable of supporting new sprouts, if the reinnervating units die before effective sprouting takes place (Stålberg and Trontelj, 1979), or if antisprouting factors exist (Gurney *et al.*, 1984). There is recent evidence pointing to a putative motor nerve growth factor released from muscle, analogous to nerve growth factor, which may be responsible for the sprouting mechanism (*see* review by Slack *et al.*, 1983). However, the preservation of the reinnervative capacity in the early stages of ALS argues against possible antisprouting factors (Swash and Schwartz, 1982), considering that this would probably produce a dying-back type of disorder and impaired reinnervation (Bradley, 1987).

Autopsy reports on SMA are scarce, specifically for the late onset variety (Kohn, 1968; Tomlinson *et al.*, 1974; Tsukagoshi *et al.*, 1979), and generally it is believed that the main lesions are in the anterior horn cells (*see* Banker, 1987). The same site of involvement and the possible mechanism of neuronopathy (Thomas, 1981) have been noted in another motor neuron disorder, ALS. Among others, this mechanism in ALS is supported by the phrenic nerve studies of Bradley *et al.* (1983), and recently by our intramuscular nerve studies (Rosales *et al.*, 1988). Although of necessity shorter lengths of the nerve were studied, this work demonstrated the loss of MFs in both proximal and distal sites. Similarly, we have advanced the possible mechanism of neuronopathy in adult-onset SMA. Pathological studies on the 'wobbler' mouse, believed to be a model of hereditary motor neuron disease (Duchen and Strich, 1968), have likewise supported this concept (Mitsumoto and Bradley, 1982, 1985; Mitsumoto, 1985). Furthermore, Lewkowicz (1979) found raised terminal innervation patterns in sampled muscles of the same animal model.

Johnson *et al.* (1973*b*) considered that the clinically observed muscle wasting in SMA was probably due to actual fibre loss, and we are of the opinion that these changes in the most remote parts of the nerves to the muscles in adult-onset SMA are probably axonal effects of a compromised perikaryon. While it has been previously expressed that afferent fibres of muscle spindles appear intact (Swash, 1982), our studies imply sensory involvement in adult-onset SMA as well. Pathological studies in the past have indicated that nonmotor systems (Marshall and Duchen, 1975; Carpenter *et al.*, 1978; Tsukagoshi *et al.*, 1979; Mukai *et al.*, 1981), including peripheral sensory nerves, may be affected in some forms of SMA (Harding *et al.*, 1982; Wilde *et al.*, 1987).

We specifically recognize some shortcomings in the methodology of this work. We limited ourselves to clinical history and examination of a few relatives in seeking family backgrounds for our cases. The difficulty in distinguishing between motor and sensory fibres in our intramuscular nerve analysis is also recognized (*see* Kakulas and Adams, 1985). Despite these caveats, not only can the quantitative analysis of intramuscular nerves provide a dynamic interpretation of the underlying disease process, but it is also able to document the pattern of nerve fibre loss in relation to the quantitatively determined muscle fibre grouping. Finally, we anticipate that this approach will find advantages in formulating associations with electromyographic parameters in the same sampled muscle.

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VOLUNTARY MUSCLE CONTROL IN NORMAL AND ATHETOID DYSARTHRIC SPEAKERS

by N. J. O'DWYER *and* P. D. NEILSON

(*From the Spastic Centre Research Unit, School of Medicine, University of New South Wales, and Department of Neurology, The Prince Henry Hospital, Sydney, Australia*)

SUMMARY

Time domain and frequency domain analyses were performed on smoothed and averaged electromyographic (IEMG) activity recorded intramuscularly from 6 muscles of the lips, tongue and jaw during speech in normal and athetoid cerebral palsy subjects. The speech IEMG waveforms in both groups were composed of slowly changing tonic activity merging with more rapidly changing phasic bursts. Significant increases both in the durations and average levels of IEMG activity in the athetoid subjects resulted in a 5- to 30-fold increase in the speech muscle energy expended by these subjects. The peak-to-peak amplitudes of the IEMG activity were significantly increased in the athetoid subjects, commensurate with their increased average levels, thus demonstrating that they could vary their muscle contraction levels over a wide range. The velocities (rates of change) of muscle IEMG activity did not differ significantly between the two groups. The velocity of the IEMG activity increased linearly with its amplitude in both subject groups, but the durations of the IEMG bursts nevertheless were highly variable. The slope of the velocity-amplitude relation in the athetoid subjects was less than half that in the normal subjects, suggesting that the frequency bandwidth of muscle activity was reduced in the athetoid subjects, despite a normal range of IEMG velocities. The frequency analysis confirmed this suggestion. The upper limit of the average frequency spectrum of voluntary muscle activity for speech was 7 Hz in the normal subjects, whereas this limit was 4 Hz in the athetoid subjects. In the normal subjects each muscle had a different frequency spectrum, whereas the spectra for the 6 muscles were remarkably uniform in the athetoid subjects, implying an abnormality in the functional organization of their muscles. The findings of this study showed clearly that the temporospatial patterns of *voluntary* muscle activity in the athetoid subjects were grossly abnormal. Since this voluntary activity was reproducible across multiple repetitions of the same speech sample, the dysarthria in these speakers may be attributed to abnormal control of voluntary activity, not to involuntary movement. The results support the view that the primary disability in cerebral palsy is a disruption of the physiological mechanisms which subserve the acquisition of motor skills.

INTRODUCTION

Several theories have been proposed concerning the pathophysiology of dysarthria in cerebral palsy. Weakness of speech muscles has been suggested to be of prime

Correspondence to: Dr N. J. O'Dwyer, Spastic Centre Research Unit, Neurology/Medicine, The Prince Henry Hospital, Sydney, NSW 2036, Australia

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importance (Ingram and Barn, 1961; Hardy, 1961, 1964, 1967; Hixon and Hardy, 1964). Abnormalities of muscle tone due either to spasticity of speech muscles (Clement and Twitchell, 1959; Ingram and Barn, 1961; Paine and Oppé, 1966; Holt and Reynell, 1967) or sustained background activity have been thought to be significant. Primitive reflexes or pathological reactions have been said to interfere with articulatory control (Mysak, 1963; Crickmay, 1966). An imbalance said to exist between positive and negative oral reactions has been thought to disrupt speech (Denny-Brown, 1958; Clement and Twitchell, 1959; Twitchell, 1961). In athetoid cerebral palsy, dysarthria has been thought to result from involuntary movements (Ingram and Barn, 1961; Paine and Oppe, 1966; Spiegel and Baird, 1968; Darley *et al.*, 1975; Brain, 1977; Rosenbek and LaPointe, 1978). For all these theories, however, there has been little experimental verification. Several of them have been described by Foley (1983) as 'cherished theories that have held sway too long'. Experimental investigations in this laboratory (Neilson *et al.*, 1979; Neilson and O'Dwyer, 1981, 1984; O'Dwyer *et al.*, 1983) have failed to support these existing theories. Instead, the results available suggest that, consequent to a motor learning deficit, a fundamental abnormality exists in the formulation of voluntary motor commands to muscles in cerebral palsy.

As part of our previous investigations, we quantified electromyographic (EMG) activity recorded from 2 lip, 2 tongue and 2 jaw muscles during 20 recitations of a test sentence by 5 normal and 5 athetoid dysarthric speakers (Neilson and O'Dwyer, 1984). In the athetoid subjects the articulation of the test sentence was disrupted intermittently and irregularly by involuntary activity, so that the timing of their total motor output for the sentence was both prolonged and variable compared with normal subjects. This involuntary activity usually occurred in the time intervals between the syllables in the test sentence, such that speech output was often halted transiently. With respect to articulation of the syllables themselves, however, the pattern of muscle activity employed for any 1 syllable by any 1 subject was very similar from one repetition of that syllable to the next. We partitioned the EMG activity associated with each of the 10 syllables in the test sentence into its reproducible and variable components. The ratios of the mean square value of the reproducible ('signal') component to the mean square value of the variable ('noise') component did not differ significantly between the 2 subject groups. (The grand mean signal-to-noise ratios for the 20 recitations of the test sentence by the normal and athetoid subjects were 19:1 and 16:1, respectively.) Thus the patterns of muscle activity employed for the articulation of individual syllables were similarly reproducible for each repetition of the syllables in the athetoid and normal subjects. The variable involuntary movements which occurred in the athetoid subjects could not therefore be the cause of their dysarthria because articulation of the syllables themselves was largely unaffected by such movements. On the other hand, the reproducible component of the EMG activity in the athetoid subjects was grossly abnormal. It was concluded that this reproducible voluntary activity, rather than variable involuntary activity, is the primary cause of athetoid dysarthria (Neilson and O'Dwyer, 1984). Thus although the speech of athetoid dysarthric speakers is indeed disrupted by involuntary movements, the underlying speech is already grossly abnormal owing to the abnormal but reproducible patterns of muscle activity which produced it.

In the present study we concentrate on this abnormal but reproducible muscle activity. The characteristics of the voluntary speech muscle activity of normal and athetoid subjects are quantified and compared. Our purpose is to eludicate the abnormalities of voluntary motor control in athetosis in order to shed light on the pathophysiological mechanisms involved.

METHODS

The experimental procedure has been described fully elsewhere (Neilson and O'Dwyer, 1981; O'Dwyer *et al.*, 1981). EMG activity was recorded intramuscularly from 6 speech muscles: orbicularis oris superioris (OOS) and depressor labii inferioris (DLI) in the lips, the anterior genioglossus (GG) and geniohyoid (GH) in the tongue and the medial (internal) pterygoid (IP) and anterior belly of the digastric (ABD) in the jaw. Muscle activity was analysed during 20 recitations of the test sentence: 'Do all the old rogues abjure weird ladies'. This sentence was composed to mimic conversational speech and to require large ranges of movement of the lips, tongue and jaw for its production (van Doorn, 1982), thereby emphasizing any abnormalities of speech in the athetoid subjects. Subjects were instructed to attempt to maintain the same prosody (rhythm, stress, intonation and loudness) in each recitation of the sentence.

Subjects

The subjects were 5 normal fluent speakers, aged 20-30 yrs, and 5 cerebral-palsied dysarthric speakers, aged 19-34 yrs. All subjects were volunteers who gave informed written consent to the experimental procedures. None of the cerebral-palsied subjects were mentally retarded, although all were severely disabled. On detailed neurological examination they were judged to be predominantly athetoid, although mild upper motor neuron signs also were present. Facial grimacing was present in all 5 subjects and their speech was grossly athetotic. Although intelligibility was not measured, none of the athetoid subjects could be understood by those unfamiliar with their speech.

Muscle signal processing

It is well known that muscles behave as low-pass filters with respect to neural input (Partridge, 1965; Poppele and Terzuolo, 1968; Rosenthal *et al.*, 1970; Bawa and Stein, 1976), that is, slow (low frequency) changes in neural activity are 'passed' (hence, low-pass) by muscles and appear in the tension output, whereas faster (higher frequency) changes in neural activity are partially or completely diminished by muscles and appear in reduced form or not at all in the tension output. This low-pass filtering, or smoothing, results in a limited frequency bandwidth of tension output from muscles. In keeping with this, a limited bandwidth also has been documented for voluntary movements (Stark, 1968; Neilson, 1972*a*; Terzuolo *et al.*, 1981; Freund, 1983), including speech (Müller *et al.*, 1981). For this reason, the EMG signals recorded in this study were smoothed prior to further analysis. This smoothing was achieved by full-wave rectification followed by low-pass filtering through two first-order filters connected in cascade (for each filter: time constant = 0.02 s; corner frequency = 8 Hz). Such smoothed EMG signals are functionally related to muscle tension and this relation is linear under isometric conditions or during contractions with constant velocity (Inman *et al.*, 1952; Lippold, 1952; Bigland and Lippold, 1954; Gottlieb and Agarwal, 1971; Lindström *et al.*, 1974; Milner-Brown and Stein, 1975; Hof and van den Berg, 1977). These signals are often described, albeit inaccurately, as 'integrated' EMG signals and will be referred to hereafter

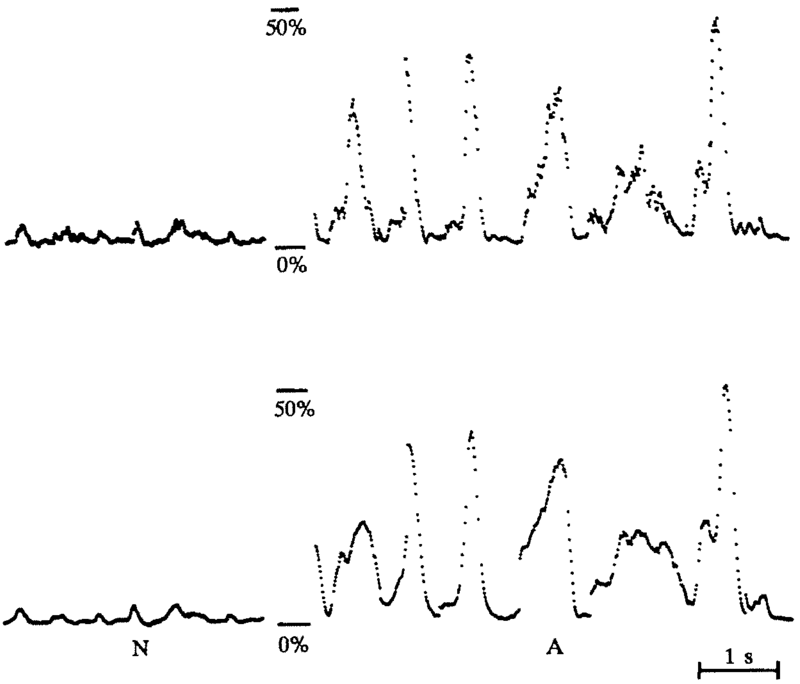

FIG. 1. Smoothed EMG (IEMG) signals recorded from the anterior belly of the digastric muscle in a normal (N) and an athetoid (A) subject. The upper traces are the signals recorded during a single recitation of the test sentence, while the lower traces are the average of 20 recitations of the sentence. Each trace consists of 10 syllable waveforms which have been concatenated together. The vertical calibrations indicate 0% and 50% of maximal voluntary contraction.

as IEMG signals. The IEMG signals were digitized at 100/s and subsequently were calibrated with respect to maximum isometric contractions recorded for each muscle (*see* O'Dwyer *et al.*, 1981, 1983, for details). For this study, one unit of IEMG will be defined as 1% of maximum voluntary contraction.

The test sentence contained 10 syllables. The IEMG activity associated with each syllable was identified for each muscle using an algorithm which excluded any activity associated with halting of speech output or with prolongation of the intervals between syllables, such as might occur, for example, due to involuntary spasms in the athetoid subjects (*see* Neilson and O'Dwyer, 1984, for details). The IEMG activity associated with each sentence was thus reduced to 10 individual 'syllable waveforms', examples of which are shown for the ABD muscle in the upper traces in fig. 1. Here the 10 IEMG syllable waveforms have been concatenated to yield a 'composite' IEMG waveform for the sentence.

We have shown previously (Neilson and O'Dwyer, 1984) that these IEMG syllable waveforms were reproducible across the 20 recitations of the test sentence and moreover, that the degree of reproducibility was comparable in the two subject groups. This reproducibility is apparent when the waveforms for a single recitation of the sentence are compared with the average waveforms for the 20 recitations, shown here for the ABD muscle in the lower traces in fig. 1. Such reproducibility of the IEMG activity indicates that the average waveforms may be regarded as valid estimates of the voluntary muscle activity of these subjects. Accordingly, the remainder of the present report will be devoted to analysis of these average waveforms.

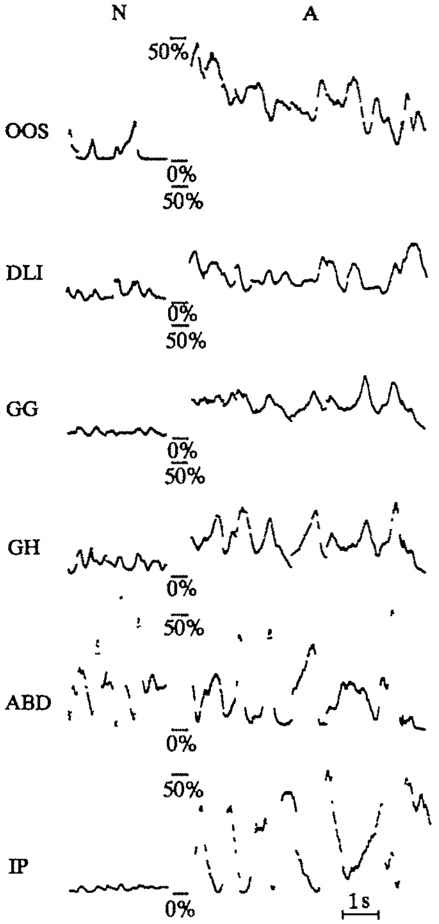

FIG. 2. IEMG activity of lip, tongue and jaw muscles averaged over 20 recitations of the test sentence in a normal (N) and an athetoid (A) subject. Each trace consists of 10 syllable waveforms concatenated together Abbreviations for muscles as in text. Muscle calibrations as in fig 1.

Waveform analysis

The average waveforms of the 6 speech muscles for each of the 10 syllables are shown for normal and athetoid subjects in fig. 2. This figure contains the complete set of IEMG waveforms analysed for these 2 subjects. We wished to quantify the differences between the groups which are illustrated by these traces. Both time domain and frequency domain characteristics of the waveforms were examined. Multiple time domain measures were made so that many features of the waveforms would be assessed, thereby maximizing discrimination between the groups and minimizing the possibility that specific abnormalities could be overlooked. Frequency analysis was applied because it has been shown previously in athetoid subjects to reveal abnormalities not evident through time domain analyses (Neilson, 1974*a*, *b*).

Time domain analysis

The average waveforms of the 6 speech muscles for each of the 10 syllables in the 5 normal and 5 athetoid subjects, a total of 600 waveforms, were analysed. It was already known (from the EMG processing described above) that the durations of the syllable waveforms were significantly prolonged in the athetoid subjects (Neilson and O'Dwyer, 1984). The following measures were made of the magnitude of each syllable waveform. root mean square value, average level, maximum and minimum values, standard deviation (SD) and peak-to-peak amplitude (maximum minus minimum). The IEMG energy, defined as the integral of the squared waveform (Doerschuk *et al.*, 1983) for each syllable, also was computed. The average waveforms were differentiated to yield waveforms which represented the rate of change of the original IEMG activity. These waveforms will be referred to as 'velocity' waveforms. The following measures were made of the velocity waveforms for each syllable: SD, root mean square value, mean rectified value, average positive and negative values and maximum positive and negative values.

Amplitude distribution histograms calculated for each of the above measures revealed that none was normally distributed. Logarithmic transformation of the measures caused the distributions to approach normality (although with a negative skew in the athetoid data) but failed to produce homogeneity of error variances between the two subject groups, as tested by Hartley's and Cochran's tests (Winer, 1971, p. 205). These tests showed that the athetoid group was significantly more heterogeneous than the normal group with respect to all factors examined. Thus, for the purposes of comparisons between subject groups, the prerequisites of parametric statistics were not fulfilled.

Some preliminary analyses were carried out within each subject group. The relations between all of the waveform measures were examined by regression analysis. The regression of each magnitude measure on each other magnitude measure and of each velocity measure on each other velocity measure was computed. Similarly, the regression of each velocity measure on each magnitude measure was computed. Each regression analysis was based on 300 pairs of data points in each group. These analyses showed that most of the measures were highly correlated within each subject group and, furthermore, that the pattern of the correlations across the measures was very similar in the two groups. It should be pointed out that high correlations are not inevitable between most of these measures, so that a considerable uniformity of IEMG waveform characteristics between the two groups was indicated by the results of these regression analyses.

In the light of these correlations between the measures, a principal components analysis (Harris, 1975; Nie *et al.*, 1975) was carried out within each group. This analysis showed that almost 90% of the variance of the waveform measures within each group could be accounted for by just 2 (uncorrelated) linear combinations of the measures; that is, by 2 independent 'factors' derived from the original measures. The factor structure was highly similar in the 2 groups. Rotation (normalized varimax criterion) of the factor matrix revealed 2 groupings of the waveform measures which had a simple substantive interpretation. One factor appeared to represent the average *level* of the IEMG activity or the tonic muscle activity, since the measures which loaded most strongly onto this factor—average level, root mean square value, maximum, minimum and energy—all have in common the fact that they are influenced directly by the average level. The other factor appeared to represent both the *amplitude* and the *velocity* of the IEMG activity or the phasic muscle activity, since the measures which loaded most strongly onto this factor—peak-to-peak amplitude, SD and the 7 velocity measures—all have in common the fact that they are influenced directly by the changes in the IEMG activity, rather than the average level. In order to make statistical comparisons between the subject groups, therefore, the measure which correlated most highly with each factor was selected to represent that factor. For both groups the selected measures were the minimum level (representing *level*) and the root mean square velocity (representing *velocity*). In addition, the peak-to-peak amplitude was selected to represent the *amplitude* of the IEMG activity because, as will be seen below, this measure provided further discrimination between the groups. Thus, 3 features of the IEMG waveforms—*level*, *amplitude* and *velocity*—were compared between the 2 subject groups.

A nonparametric or distribution-free analysis of variance (Bradley, 1968, p. 138) was applied to the selected measures. In this analysis there was one group factor, to which the Wilcoxon rank-sum test (statistic: W_n) was applied and there were 2 repeated measures factors—muscles and syllables—to which the Friedman test (statistic: χ_r^2) was applied. Since the principal components analysis indicated a 'structure' comprising 2 independent factors in the waveform measures, the critical significance level for each test was adjusted to $0.05/2 = 0.025$ using the Bonferroni approach (Harris, 1975, p. 98). This procedure maintained the overall experimentwise error rate conservatively at 0.05.

Frequency domain analysis

Whereas the time domain analysis was based on each of the 10 syllable waveforms for each muscle, the frequency analysis was performed on the composite waveform for the whole sentence, consisting of the concatenated syllable waveforms (as illustrated in fig. 2). This was determined by the requirements of the frequency analysis technique (Bendat and Piersol, 1966) regarding length of data sample. A five-point smoothing procedure was applied to each composite waveform in order to eliminate any abrupt changes in IEMG level introduced by concatenating the syllable waveforms. The average waveforms for the 6 muscles in the 10 subjects, a total of 60 waveforms, were analysed.

The frequency power spectrum of each muscle waveform was computed by taking the Fourier transform of the autocorrelation function. The spectrum was estimated every 0.5 Hz from 0 Hz to 10 Hz (giving 21 frequencies), with a resolution bandwidth of 1 Hz. The spectrum describes the total power (= mean square value) of the IEMG signal and its distribution across the 21 frequencies. It is the distribution across frequency which is of primary interest here, rather than the absolute level of power, which is adequately assessed by the magnitude measures. Therefore the power spectral density of each muscle waveform also was computed. The spectral density is the 'relative power spectrum', that is, the power at each frequency expressed as a proportion of the total power in the spectrum. Thus the total area under a spectral density curve is unity. The power spectral density emphasises the distribution across frequency of the IEMG power, rather than its absolute level, thereby facilitating comparison between signals having different levels of total power. Statistical comparison of the groups will be made only on spectral density. Of particular interest in this comparison will be the frequency bandwidth of the IEMG signals, that is, the range of frequencies over which the power is distributed.

Power spectra also were computed for the IEMG velocity waveforms. Although all changes in IEMG activity are described by the spectra of the original IEMG signals, the spectra of the velocity waveforms emphasise the faster changes in activity. Thus the velocity spectra predominantly reflect changes in IEMG activity associated with individual syllables in the test sentence, rather than more 'tonic' changes with a time course spanning several syllables or the entire sentence.

The nonparametric analysis of variance (Bradley, 1968, p. 138) was also employed for the comparison of the spectral densities. The analysis had 1 group factor, as before, while the repeated measures factors in this instance were muscles and frequencies. Only the effects involving the frequency factor were of interest in this analysis. The main effects of groups and muscles were not relevant since the sum of the spectral densities for each group and for each muscle necessarily were identical. This is because spectral densities for each muscle in each subject sum to unity.

The criteria for evaluating experimental data with regard to departures from the homogeneity and normality assumptions of parametric tests are rather poorly defined (Bradley, 1968), so that decisions regarding the application of nonparametric versus parametric tests are seldom clearcut. It is worthy of note, therefore, that for all tests in the present study using a nonparametric analysis of variance, very similar statistical effects could be obtained using parametric analysis of variance (Winer, 1971) of the same data at the same level of significance.

RESULTS

Examination of the traces for the normal and athetoid subject in fig. 2 demonstrates that their IEMG signals contain both slowly changing tonic activity and more rapidly changing phasic bursts. The duration, magnitude, velocity and 'shape' of the IEMG bursts vary from moment to moment in an irregular manner, with no 2 bursts being exactly the same. Moreover, the 2 subjects appear similar

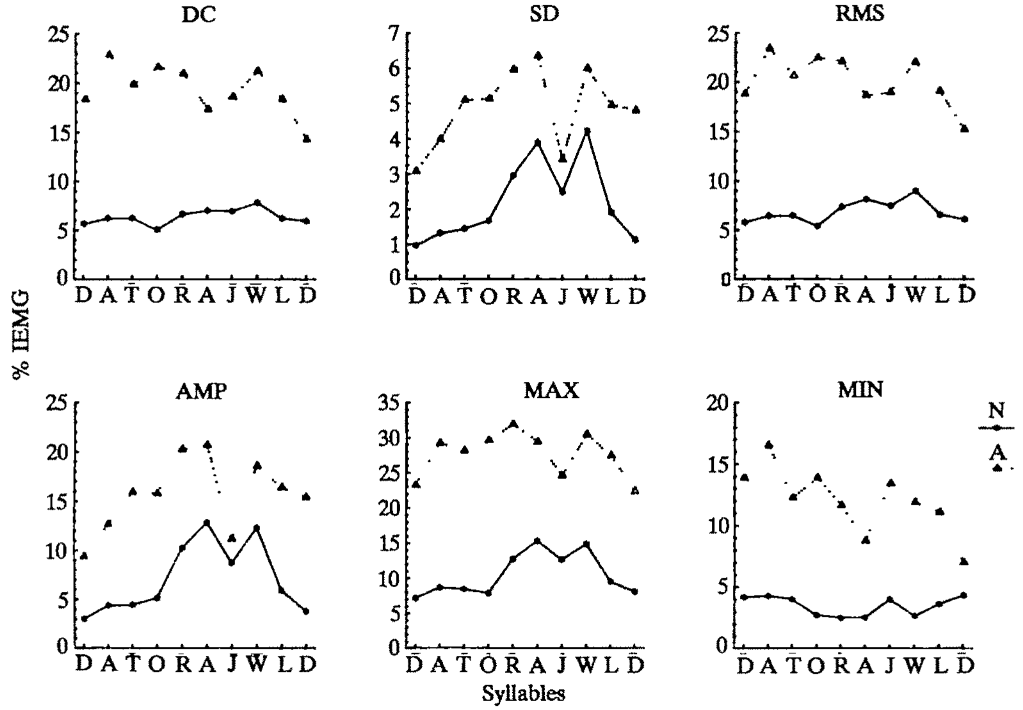

FIG. 3. Profiles of the mean value (averaged across muscles) for each syllable waveform, for 6 measures of IEMG magnitude in the normal (N) and athetoid (A) groups. The measures are: average level (DC), standard deviation (SD), root mean square value (RMS), peak-to-peak amplitude (AMP), maximum value (MAX) and minimum value (MIN). Abbreviations for the 10 syllables are the first letter of each syllable, indicated by capitals as follows: Do All The Old Rogues AbJure Weird LaDies. All measures are in units of %IEMG

in these respects. These IEMG characteristics were observed in all subjects in both groups.

Time domain measures

Increased durations of the waveforms in the athetoid subjects, which were reported previously, are evident in the traces in fig. 2. An overall increase in the magnitudes (level and amplitude) of IEMG activity also is apparent in the athetoid subjects. This is borne out by all of the measures of waveform magnitude (fig. 3).

When the effects of duration and magnitude are combined, as reflected in the IEMG energy, a striking difference between the groups becomes apparent (fig. 4). In order to articulate the test sentence, the athetoid subjects employed, on average, about 20 times as much energy in lip muscles and nearly 30 times as much energy in tongue muscles as normal subjects. The energy cost in jaw muscles was not as high, about a 5-fold increase.

The analysis of variance of the minimum level confirmed that the overall increase in the athetoid subjects on this measure, evident in fig. 3, was indeed significant

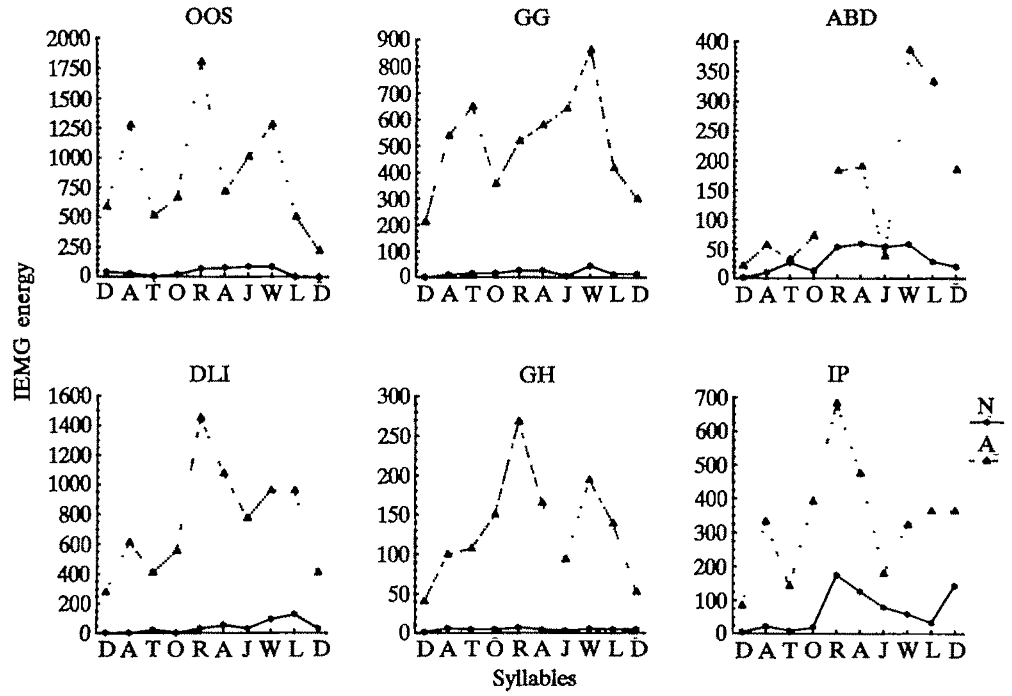

FIG. 4. Profiles of mean IEMG energy for the 10 syllable waveforms in each of the 6 muscles in the normal (N) and athetoid (A) subject groups. Note differences in scaling across the 6 muscles. Abbreviations for muscles as in text and for syllables as in fig 3. Energy is in squared units of %IEMG.

(group effect: $W_n = 15$, $n_1 = n_2 = 5$; $P < 0.01$). However, important other differences between the groups also were revealed by this analysis. There was a significant variation in the minimum level across the muscles (muscle effect: $\chi_r^2 = 17.9$, degrees of freedom = 5; $P < 0.01$) and this variation was significantly different in the 2 subject groups (group × muscle interaction effect: $\chi_r^2 = 13.6$, df = 5; $P < 0.025$). This interaction appears to reflect a group difference between lip and tongue muscles on the one hand, where the level was increased 4- to 6-fold in the athetoid subjects and jaw muscles on the other hand, where the increase was less than 2-fold. This is similar to the pattern noted above in the profiles of IEMG energy (fig. 4). There was also a significant variation in the minimum level across the syllables in the test sentence (syllable effect: $\chi_r^2 = 20.3$, df = 9; $P < 0.025$) and again this variation was significantly different in the 2 subject groups (group × syllable interaction effect: $\chi_r^2 = 19.1$, df = 9; $P < 0.025$). This interaction points to a group difference in the temporal pattern of the level of muscle activity across the sentence. The variation of the minimum level across the syllables had a pattern which varied significantly across the muscles (muscle × syllable interaction effect: $\chi_r^2 = 101.4$, df = 45; $P < 0.01$). The 2 subject groups, however, did not differ significantly in this respect (group × muscle × syllable interaction effect: $\chi_r^2 = 57.0$, df = 45; ns). It

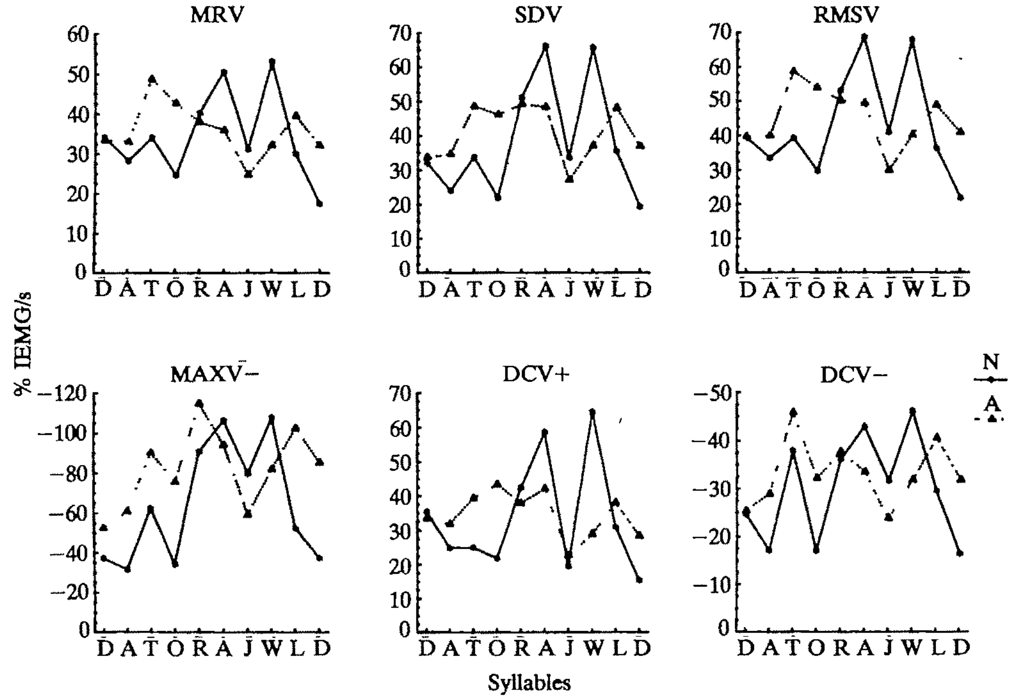

FIG. 5. Profiles of the mean value (averaged across muscles) for each syllable waveform, of 6 measures of IEMG velocity in the normal (N) and athetoid (A) groups The measures are: mean rectified velocity (MRV), standard deviation of velocity (SDV), root mean square velocity (RMSV), maximum negative velocity (MAXV−), average positive velocity (DCV+) and average negative velocity (DCV−). Velocity is in units of %IEMG/s. Abbreviations for muscles as in text and for syllables as in fig. 3

may be noted that essentially the same statistical effects could be found on all of the level measures (average level, root mean square value, maximum and minimum levels and energy) of the IEMG waveforms.

The analysis of variance of the peak-to-peak amplitude of the IEMG activity confirmed that the overall increase in the athetoid subjects on this measure, evident in fig. 3, also was significant (group effect: $W_n = 15$, $n_1 = n_2 = 5$; $P < 0.01$). However, no other significant group differences were observed on this measure. The statistical effects observed on the SD of the IEMG activity, the other amplitude measure of the waveforms, were similar to those for the peak-to-peak amplitude.

In contrast to the durations and magnitudes, all measures of velocity of the IEMG activity showed considerable overlap between the 2 subject groups (fig. 5). Moreover, even for maximum velocities, this overlap applied across all of the lip, tongue and jaw muscles (fig. 6).

The analysis of variance of the selected velocity measure confirmed the similarity of the groups with respect to overall velocities of the IEMG activity but revealed important group differences in the pattern of velocities. The lack of a significant

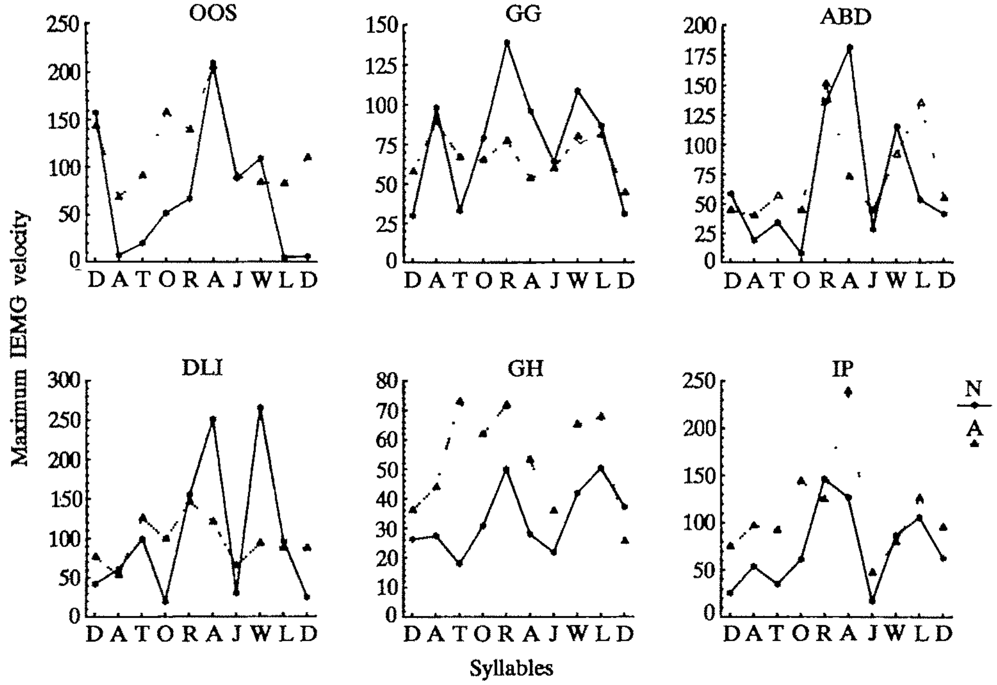

FIG. 6 Profiles of the mean value of the maximum positive IEMG velocity for the 10 syllable waveforms in each of the 6 muscles in the normal (N) and athetoid (A) subjects. Note differences in scaling across the 6 muscles. Velocity is in units of %IEMG/s. Abbreviations for muscles as in text and for syllables as in fig 3.

overall group difference in the root mean square velocity, evident in fig. 5, was confirmed (group effect: $W_n = 24$, $n_1 = n_2 = 5$; ns). Furthermore, there was no significant variation in the velocity across the 6 muscles (muscle effect: $\chi_r^2 = 5.3$, df = 5; ns) and the 2 subject groups were similar in this respect (group × muscle interaction effect: $\chi_r^2 = 1.6$, df = 5; ns). The variation of the velocity across the syllables, on the other hand, was significant (syllable effect: $\chi_r^2 = 31.1$, df = 9; $P < 0.01$) and this variation was significantly different in the 2 subject groups (group × syllable interaction effect: $\chi_r^2 = 25.8$, df = 9; $P < 0.01$). This interaction points to a group difference in the temporal pattern of the velocity of muscle activity across the sentence. Furthermore, the variation of the velocity across the syllables had a pattern which varied significantly across the muscles (muscle × syllable interaction effect: $\chi_r^2 = 81.3$, df = 45; $P < 0.01$) and these patterns were significantly different in the 2 subject groups (group × muscle × syllable interaction effect: $\chi_r^2 = 68.5$, df = 45; $P < 0.025$). This latter interaction points to a difference between the groups in the temporospatial pattern of velocities across syllables and muscles. Similar statistical effects could be observed for all of the velocity measures.

It was noted earlier that most of the level, amplitude and velocity measures were highly intercorrelated within each subject group. While the level and

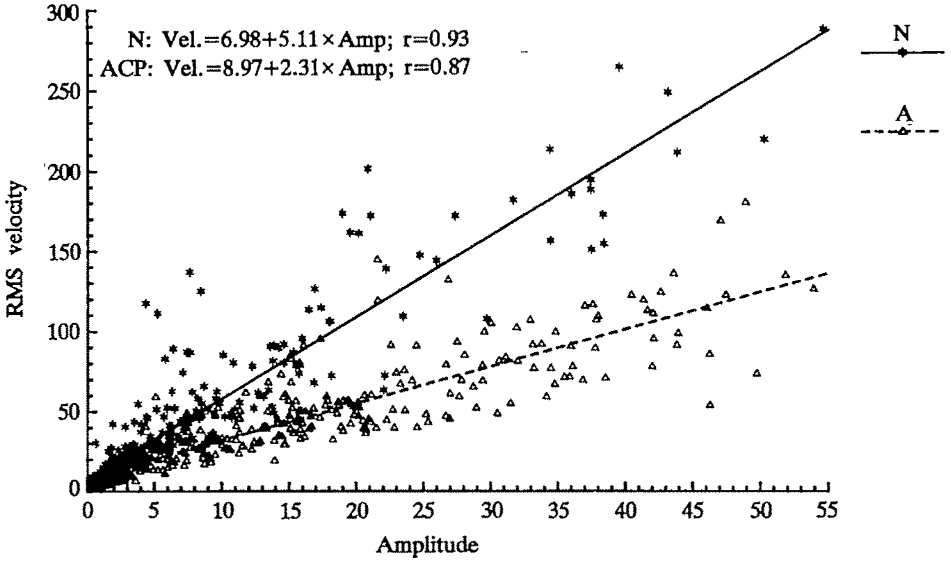

FIG. 7. Scatter plot of root mean square velocity against peak-to-peak amplitude for the normal and athetoid subject groups. The plot contains 600 pairs of data points, 300 for each subject group. The regression lines fitted to the data for each group are shown and the corresponding regression equations and correlation coefficients also are presented above the plot. N = normal subjects; A/CP = athetoid subjects. Velocity and amplitude are in units of %IEMG/s and %IEMG, respectively.

amplitude measures are increased in the athetoid subjects, however, a parallel increase in their velocity measures is not observed. This reflects the fact that the slopes of the regressions between the velocity measures on the one hand and the level and amplitude measures on the other, are reduced in the athetoid subjects. A scatter plot of the root mean square velocity against peak-to-peak amplitude for the 2 groups (fig. 7) illustrates this point. The regression equations fitted to the data for each group also are shown. The coefficients of determination (r^2) show that 86% and 76% of the variance of the root mean square velocity can be accounted for by the peak-to-peak amplitude in the normal and athetoid subjects, respectively. Clearly, the velocity of the IEMG activity increases with its amplitude in both groups, but the rate of this increase in the athetoid subjects is less than half that in the normal subjects, reflecting the lower slope (regression coefficient) of their relation between these measures. The higher amplitudes in the athetoid group, however, serve to offset their slower velocity increase, so that, overall, the velocities do not differ between the groups. The simplest explanation for the reduced slope in the athetoid subjects, without altering the level of correlation, is that the frequency bandwidth of their IEMG waveforms is reduced—for sine waves, for example, the slope of the velocity-amplitude relation increases linearly with frequency. This explanation will be confirmed below.

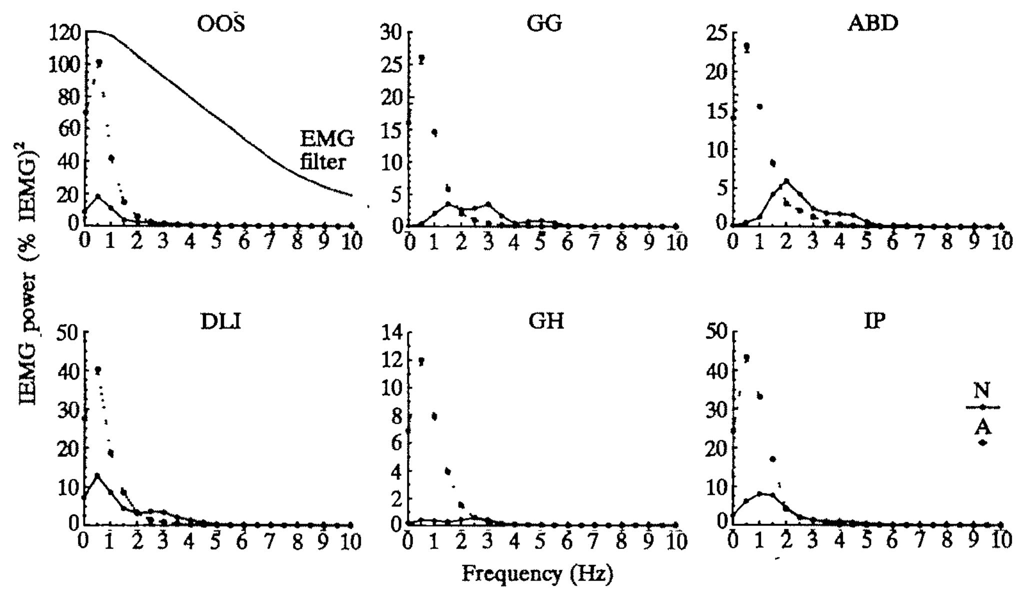

FIG. 8. Mean power spectra of the IEMG waveforms for the 6 muscles in the normal (N) and athetoid (A) subject groups. The bandpass of the EMG filter is illustrated with the spectra for OOS. Each data point represents the mean square value of the IEMG activity in a 0.5 Hz interval centred about each point on the frequency scale. Power is in units of %IEMG squared. Note differences in scaling across the muscles. Abbreviations for muscles as in text.

Frequency domain measures

The frequency bandpass of the EMG filter is illustrated by the trace labelled 'EMG filter' in fig. 8. If the input to the filter were a signal with equal power at all frequencies, then the output from the filter (after scaling to the units of measurement shown) would have the power spectrum shown. At 0 Hz the filter has no effect, so that the output power is identical to the input power. With increasing frequencies, however, the filter has a progressively greater effect, so that by 10 Hz the output power is reduced to approximately a sixth (20/120) of the input power. At still higher frequencies (not shown), the output power is progressively reduced towards zero. It can be seen that the frequency bandwidth of all the IEMG waveforms in both subject groups was well within the filter bandpass and so was not limited by the EMG filter. It follows from this that any similarities or differences in the IEMG waveforms between the 2 groups cannot be attributed to differential effects of the EMG smoothing on either group. Thus the IEMG spectra may be taken as truly reflecting the bandwidth of voluntary speech muscle activity in the 2 groups.

The greater overall spectral power of the IEMG activity in the athetoid compared with the normal subjects, evident in fig. 8, reflects the increased magnitude of their muscle activity. The focus of interest here, however, is the frequency effects, which are better illustrated by the power spectral density of the IEMG waveforms

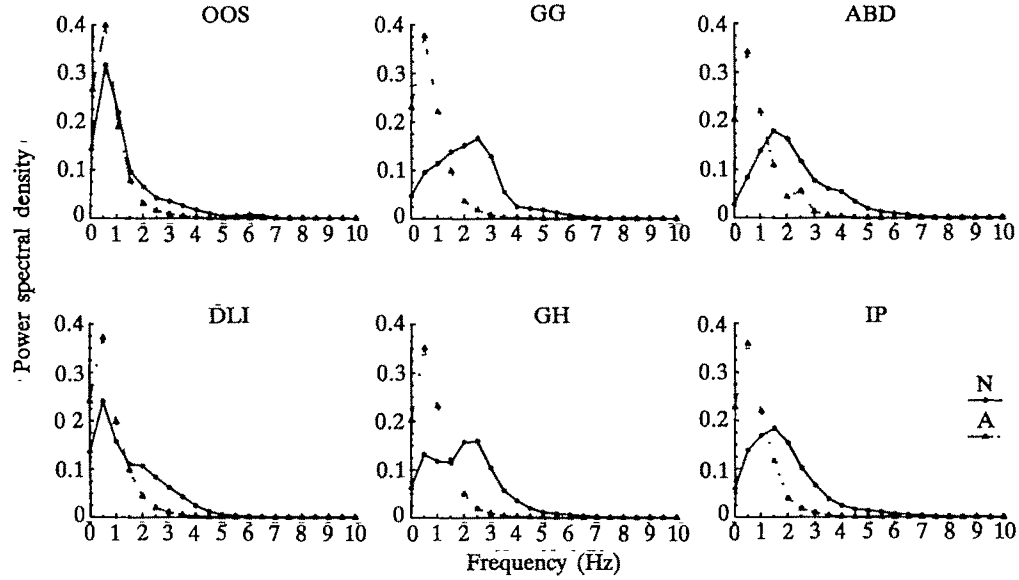

FIG. 9. Mean power spectral density of the IEMG waveforms for the 6 muscles in the normal (N) and athetoid (A) subjects. Spectral density is the power at each frequency expressed as a proportion of the total power in the spectrum. Each value represents the proportion of the IEMG power in each 0.5 Hz interval centred about each point on the frequency scale. Abbreviations for muscles as in text.

(fig. 9). The athetoid subjects have the peak in their spectral density curves at 0.5 Hz, with power dropping to low values by 2 Hz and becoming negligible above 3-3.5 Hz. It is particularly noteworthy that this pattern is the same in all 6 muscles. In the normal subjects, on the other hand, the shape of the spectral density curve is different in each muscle and may have broad or multiple peaks. Moreover, there is power in all muscles up to higher frequencies, at least 5 Hz, so that there is a wider bandwidth of muscle activity. The upper limit of IEMG power in the average spectrum for any muscle was 7 Hz in the normal subjects, while in the athetoid subjects this limit was 4 Hz.

These group differences were confirmed by the analysis of variance. The variation of the spectral density across frequencies was significant (frequency effect: $\chi_r^2 = 98.7$, df = 20; $P < 0.01$) and this variation differed significantly between the 2 subject groups (group × frequency interaction effect: $\chi_r^2 = 90.9$, df = 20; $P < 0.01$). This interaction indicates a group difference in the overall distribution of IEMG power across frequencies. Furthermore, the 'shape' of the spectral density curves varied significantly across the muscles (muscle × frequency interaction effect: $\chi_r^2 = 220.3$, df = 100; $P < 0.01$) and these shapes were significantly different in the 2 subject groups (group × muscle × frequency interaction effect: $\chi_r^2 = 177.2$, df = 100; $P < 0.01$).

The difference in bandwidth of muscle activity between the 2 subject groups is further emphasized by the spectra of the IEMG velocity waveforms (fig. 10). While

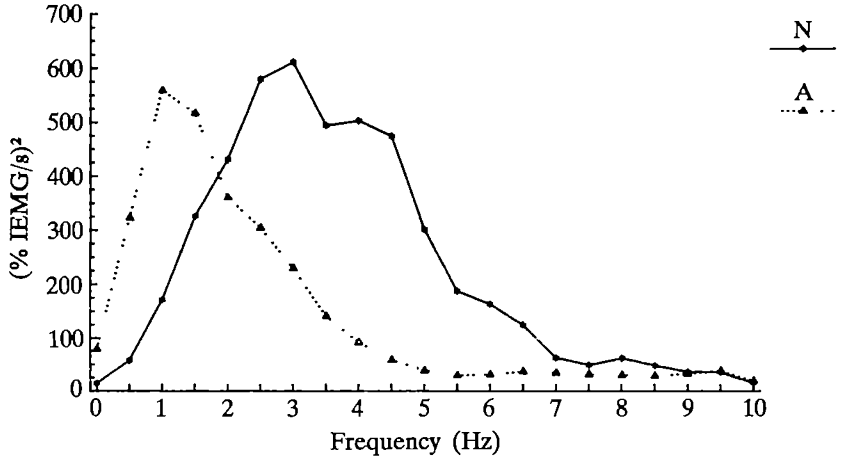

FIG. 10. Mean power spectra of the velocity of the IEMG waveforms across the 6 muscles in the normal (N) and athetoid (A) subject groups. Each data point represents the mean square value of the IEMG velocity in 0.5 Hz intervals centred about each point on the frequency scale Power is in units of (% IEMG/s)².

the shapes of the velocity spectra are not dissimilar in the 2 groups, the spectrum is broader and clearly shifted towards higher frequencies in the normal subjects. The median power frequency (that is, the frequency above and below which there is equal power) can be estimated at 1.6 Hz in the athetoid subjects and at 3.4 Hz, about twice as high, in the normal subjects.

DISCUSSION

The principal components analysis carried out on the multiple measures of magnitude and velocity of the IEMG waveforms indicated that there was a considerable uniformity of these waveform characteristics between the 2 groups. It follows that the IEMG bursts *per se* must share many characteristics in common in the 2 groups. The salient features of the IEMG bursts for the two groups are depicted in fig. 11. The waveform in the athetoid subjects consists essentially of a normal waveform which has been scaled up in magnitude (level and amplitude) and duration without altering the velocity; the frequency, which is related to the inverse of duration, is reduced. The increased amplitude of the IEMG burst in the athetoid subjects shows clearly that there is no impairment in their ability to modulate their IEMG activity over a wide range. Indeed, the depth of modulation appears appropriate to the increased level of activity. It is the increase in the average level of muscle activity, therefore, which appears to be a primary abnormality in the athetoid subjects. Increased levels of muscle activity also have been observed during arm movements in athetoid subjects (Hallett and Alvarez, 1983). The reduced frequency of the IEMG burst cannot be attributed to a limitation in the rate of change of IEMG activity. Neither positive nor negative

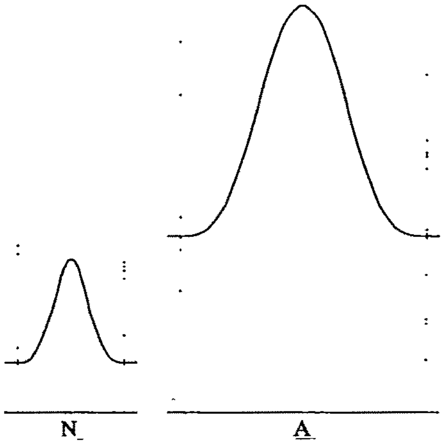

FIG. 11. Hypothetical speech muscle IEMG bursts for the normal (N) and athetoid (A) group. The relative scaling of these waveforms reflects the ratios of the grand mean values of the maximum and minimum levels, peak-to-peak amplitudes and durations for the syllables in the 2 subject groups Horizontal lines indicate the zero baseline. Vertical dotted lines indicate the boundaries of the syllable waveforms.

velocities differed between the groups (*see* figs 5, 6), so that neither activation nor relaxation, respectively, of muscle activity showed any impairment during this speech task. Therefore, the reduced frequency bandwidth of muscle activity appears to be another primary abnormality in the athetoid subjects. This reduced bandwidth indicates a reduction in IEMG waveform complexity in the athetoid compared with the normal subjects.

A correlation between velocity and amplitude, as observed for the IEMG activity in the present study, has been widely reported for human movements (e.g., Kuehn and Moll, 1976; Freund and Büdingen, 1978; Viviani and Terzuolo, 1982; Patla *et al.*, 1985; Milner, 1986). Kuehn and Moll (1976) documented this relation in movements of the lips, tongue and jaw during speech. Freund and Büdingen (1978) found that the relation applied in isometric muscle tensions, as well as in movements, of the human forefinger. The relation has been called the Isochrony Principle, whereby the velocity of movement increases approximately linearly as a function of its amplitude so as to keep the duration of the movement approximately constant (Viviani and Terzuolo, 1982). Consistent with this principle, Freund and Büdingen (1978) found that in addition to the duration of the isometric contraction itself, the duration of the associated EMG burst also remained approximately constant. In the present study, however, despite the presence of a linear relation between velocity and amplitude, it is clear from the pattern of slowly changing tonic activity merging with more rapidly changing phasic activity (fig. 2) that the durations of the IEMG bursts were far from constant, even approximately, in either subject group. The presence of an intercept in the regression equation (*see* fig. 7) in fact implies a small increase in duration with increasing amplitude and indeed, some of the studies cited above which reported a linear increase in velocity with increasing amplitude also found a smaller linear increase in duration with increasing amplitude. Thus a linear velocity-amplitude relation does not necessarily imply a constant duration and on the evidence of the present study, may be present even with wide variations in duration. In fact, we have found velocity and amplitude to be correlated even in signals composed of (low-pass) filtered random numbers. Moreover, by multiplying 2 such signals together, we have obtained correlations between velocity and amplitude of the same order as in the IEMG signals. The small effects on duration arising from a small intercept in the regression equation seem inadequate to account for the degree of variability in duration occurring in such signals. It would appear, therefore, that inferences concerning duration cannot reliably be made on the basis of a linear correlation between velocity and amplitude.

The constancy of durations observed by Freund and Büdingen (1978) is probably attributable to the fact that their study dealt with 'the fastest possible voluntary contractions', since fast movements tend to maintain a constant duration (Freund, 1983). Indeed, the notion of constancy of contraction time and EMG burst duration, if valid, may be limited to such movements. The linear velocity-magnitude relation itself, however, clearly is not limited in this way to the fastest movements but may apply in relatively slow movements as well. The presence of the relation in athetoid subjects, in particular, shows this to be the case, since the speech movements of athetoid subjects have been shown to be slower than normal (Kent and Netsell, 1978; van Doorn, 1983).

The constancy of contraction duration observed by Freund and Büdingen (1978) is of interest in its own right. In effect, what was observed in that study was a fixed time constant, or equivalently, a fixed frequency (frequency being a function of the inverse of duration) for each contraction, irrespective of its amplitude or velocity. Since these were the fastest possible contractions, it follows that the upper limit of human performance is determined not by its velocity but rather, by its time constant or its frequency. In the present study, the reduced slope of the velocity-amplitude relation in the athetoid subjects indicates that, in general, for any given amplitude of IEMG activity their velocity is lower than in the normal subjects (fig. 7). This implies that the durations of their IEMG bursts generally are prolonged (fig. 2) and this prolongation is reflected in the reduced frequency bandwidth of their IEMG activity (figs 8-10). Thus despite the fact that the overall velocities (rates of change) do not differ between the groups, the IEMG activity in fact is slower in the athetoid than in the normal subjects. These considerations demonstrate that it is important to distinguish between velocity and frequency (or time constant) in evaluating motor performance. It is apparent that normal velocities may be observed in the presence of a reduced performance bandwidth.

The most important feature of the IEMG spectra in the athetoid subjects, apart from their reduced bandwidth, was their remarkable uniformity across the 6 speech muscles. The normal subjects, on the other hand, showed a different spectrum for each muscle (figs 8, 9). It is evident from the normal spectra that an overall reduction in bandwidth alone, without any change in spectral shape, could not account for the spectra in the athetoid subjects. Therefore, while the bandwidth reduction itself reveals a reduced waveform complexity in the athetoid subjects, the uniformity of their muscle characteristics reveals an additional impairment in the functional organization of their muscles for speech. Taken together, the bandwidth reduction and spectral uniformity suggest simplified control characteristics both within muscles and in the coordination between muscles.

This study has demonstrated unequivocally that the temporal patterns of activity within muscles and the spatial patterns of activity across muscles are grossly abnormal in the athetoid subjects. Three sets of findings point clearly to this conclusion: (1) the differences between the subject groups at an individual muscle level encapsulated in fig. 11; (2) the differences between groups in muscle waveform complexity and coordination between muscles shown by the reduced bandwidths and spectral uniformity of the athetoid subjects; (3) the differences between groups in the temporospatial patterns of the level and velocity of the IEMG activity which were indicated by the interaction effects in the analyses of variance. It is apparent that the selection of muscles to be activated or inhibited and the timing of the contraction of each muscle relative to the contraction of other muscles is poorly controlled in the athetoid subjects. It is not uncontrolled, however, since the temporospatial patterns of muscle activity, while grossly abnormal, nevertheless were reproducible across multiple repetitions of the test sentence (Neilson and O'Dwyer, 1984). The significance of this latter result cannot be emphasized too strongly, since it demonstrates that the dysarthria in these athetoid subjects does not result from their involuntary, 'athetoid' movements.

Apart from their inappropriate temporospatial patterns of muscle activity, the outstanding abnormalities in the athetoid subjects were their increased average levels and reduced frequency bandwidths of muscle activity. Humphrey and Reed (1983) pointed out that increased average levels of muscle activity occur as part of increased tonic coactivation of agonist and antagonist muscles. This serves to increase the stiffness (*see* Houk and Rymer, 1981) of the body segment, thereby stabilizing the movement or posture in the presence of external perturbations. In line with this view, Abbs and Gracco (1984) reported an overall increase in lip muscle activity in response to expected (but not unexpected) external perturbations of the lip during speech. Of course, as noted by Humphrey and Reed (1983), this strategy need not be confined to external perturbations since cocontraction of antagonist muscles may be prominent in the early phases of the learning of skilled movements. 'Thus, during motor learning, the co-contraction system may be used to reduce the magnitude of movement errors that are produced by inappropriate commands from the joint-movement control system' (Humphrey and Reed, 1983, p. 371). Since the athetoid subjects under study here manifested no inability to relax muscles during ongoing speech (Neilson and O'Dwyer, 1981) or to modulate their level of activity (*see* above), their increased average levels of muscle activity may represent an attempt to resist or at least minimize the deviations from their intended movements arising from their own involuntary and/or inappropriate voluntary activity. A similar strategy also might be operative in stutterers, where increased levels of muscle activity, not attributable to an inability to relax the muscles, can be observed in association with their dysfluent utterances (e.g., Guitar *et al.*, 1988).

Variations in frequency bandwidth of motor activity are well recognized in normal subjects, where the bandwidth of performance reflects the level of skill. In tracking tasks, for example, the frequency bandwidth over which subjects can accurately follow a target increases with training (McRuer, 1980). The reduced bandwidth of muscle activity in the athetoid subjects in the present study, therefore, can be interpreted in terms of a reduced level of skill in controlling their own motor system. The spectral uniformity of their speech muscles, moreover, is in keeping with this interpretation. This bandwidth reduction in the frequency domain can be related in the time domain to the trade-off relation between the speed and accuracy of movements known as Fitts' law, after Fitts (1954). According to this law, which is widely recognized in normal human movement (*see*, e.g., MacKay, 1982; Meyer *et al.*, 1982), increased accuracy is attained by reducing the speed of movement and so allowing more time for correction of errors generated during each movement. With practice in a task, however, both the speed and accuracy of movement are increased (*see*, e.g., MacKay, 1982). Thus in the athetoid subjects under study here, the slowing of muscle activity shown by their reduced bandwidth could represent an attempt to improve the accuracy of their movements. A reduction in bandwidth of voluntary activity also can be seen in spastic cerebral-palsied subjects (Neilson, 1972*b*) and in parkinsonian subjects (Stark, 1968; Eidelberg and Dunmire, 1973) and could be interpreted in a similar manner.

It is clear from the considerations outlined above that the increased average levels and reduced bandwidths of muscle activity in the athetoid subjects both are characteristics of motor activity which normally are associated with the early phases of the learning of skilled movements. The presence of these characteristics in the athetoid subjects, therefore, is consistent with previous proposals (Neilson and O'Dwyer, 1981, 1984; O'Dwyer *et al.*, 1983) that the primary abnormality underlying the motor disability in cerebral palsy is a disruption of the physiological mechanisms which subserve motor learning. Specifically, we have postulated an inability to learn the relationships between motor commands to muscles and the resulting perceptual consequences of those motor commands. This learning can be seen as forming internal 'models' of these perceptual-motor relationships. Knowledge of such relationships is implicit in skilled motor behaviour since accurate control of the perceptual consequences of movements is what constitutes purposive activity. The postulated deficit in the mechanisms of motor learning necessarily is present in the immature nervous system in cerebral palsy, predating much of the normal period for motor learning, so that cerebral-palsied individuals are unable to progress beyond the earliest stages of motor skill acquisition. This view of cerebral palsy arose out of experimental results from the studies cited above, as well as other studies (Neilson *et al.*, 1979; Neilson and McCaughey, 1982), which failed to support existing accounts of the pathophysiological mechanisms of cerebral palsy. While cerebral-palsied subjects may appreciate precisely the intended perceptual consequences of their movements, their impaired perceptual-motor models result in the production of inappropriate motor commands to achieve the perceptual goals. These inappropriate motor commands are manifested as abnormal temporospatial patterns of muscle activity, as observed in the present study. In the case of speech muscles, the result is unintelligible or poorly intelligible dysarthric speech. The reduced frequency bandwidths and uniform spectra, together with the increased levels of muscle activity, may thus be seen as secondary manifestations of the underlying disorder in perceptual-motor modelling.

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SCHWANN CELL DEGENERATION INDUCED BY DOXORUBICIN (ADRIAMYCIN)

by JOHN D. ENGLAND,[1] EDWARD K. RHEE,[1] GERARD SAID[2] *and* AUSTIN J. SUMNER[1]

(*From the* [1]*Department of Neurology, Hospital of the University of Pennsylvania, Philadelphia, USA, and* [2]*Department of Neurology, University of Paris, CHU Kremlin-Bicêtre, Paris, France*)

SUMMARY

Doxorubicin is an anthracycline antineoplastic antibiotic that acts at the cell nucleus by intercalating between base pairs of DNA, thus inhibiting DNA-directed mRNA synthesis. Intraneural microinjection of 0.19–0.38 μg of this substance into rat sciatic nerve results in a delayed subacute demyelination that is secondary to focal Schwann cell degeneration. Remyelination eventually occurs but is not complete until at least days 60–75 postinjection. Toxic Schwann cell disorders produced by agents such as doxorubicin may serve as useful models in understanding the pathogenesis of human demyelinative neuropathies.

INTRODUCTION

Segmental demyelination is a feature common to many peripheral nerve disorders. It is a major feature of certain inherited and acquired neuropathies. Although the major pathological and electrophysiological features in various types of 'demyelinative neuropathies' have been well described (Asbury and Johnson, 1978; Sumner, 1984), the basic mechanisms underlying primary segmental demyelination are poorly understood. A primary abnormality of the Schwann cell or myelin is often presumed, but direct evidence of this is usually lacking; therefore any agent that produces primary segmental demyelination by an unequivocal direct action on Schwann cells is worthy of intensive investigation.

Doxorubicin is an anthracycline antineoplastic antibiotic that acts at the cell nucleus by intercalating between base pairs of DNA, thus inhibiting DNA-directed messenger RNA synthesis (Pigram *et al.*, 1972; Di Marco, 1975; Arcamone, 1981*a*). Using the technique of intraneural microinjection we have discovered that this agent can produce profound demyelination of peripheral nerve secondary to a direct intoxication of Schwann cells. These findings form the basis of this report.

Correspondence to: Dr Austin J. Sumner, Department of Neurology, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA.

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METHODS

Intraneural injection

Male Wistar rats (350–400 g) were used in these experiments. All procedures were carried out under anaesthesia by intraperitoneal injection of a mixture of 110 mg/kg ketamine, 13.4 mg/kg xylazine and 0.05 mg/kg atropine. This was supplemented if necessary by inhalation anaesthesia with 1–4% halothane in oxygen. The sciatic nerve and its major branches were exposed by an aseptic surgical incision from the sciatic notch to below the popliteal fossa. Using a micrometer syringe, 30 μl of the appropriate concentration of doxorubicin (Adriamycin) was injected into the subperineurial region of the single fascicle of the tibial nerve. The entire procedure was performed under an operating microscope. The following concentrations of doxorubicin were injected: (1) 30 μl of 6.25 μg/ml doxorubicin (0.19 μg) into 20 nerves; (2) 30 μl of 12.5 μg/ml doxorubicin (0.38 μg) into 25 nerves; and (3) 30 μl of 100 μg/ml doxorubicin (3 μg) into 35 nerves.

Electrophysiological studies

These were performed on all animals immediately before injection, immediately after injection, then at daily intervals until 20 days, and finally every 2–5 days until observations were complete. The compound muscle action potentials (CMAPs) from the small muscles of the foot were recorded using a platinum tipped steel recording electrode (Grass E2) placed subcutaneously on the plantar surface and an indifferent electrode inserted into the second digit. The sciatic nerve was stimulated by two pairs of these same electrodes placed percutaneously alongside the nerve at the sciatic notch and at the ankle (i.e., above and below the injection site). Square-wave pulses (0.2 ms) were delivered through an isolated voltage stimulating device (type MK-IV) triggered by a Digitimer at 1 s intervals. Care was taken to deliver supramaximal stimuli and the CMAP was amplified and displayed on one beam of a Tektronix storage cathode ray oscilloscope. Permanent photographic records were made of superimposed images. The latency of the CMAP was measured from the stimulus artefact to the beginning of the first negative deflection and the amplitude from baseline to negative peak. The ratio of the amplitude of the CMAP evoked by proximal (sciatic notch) stimulation to the amplitude of the CMAP evoked by distal stimulation was computed in each instance. Since the injection site was between these two sites of stimulation, the above noted ratio is a convenient measure of focal conduction block and/or dispersion. Additionally, the distance between the two sites of stimulation was measured along the skin surface with the leg fully extended and a segmental nerve conduction velocity was calculated. Limb temperature was carefully maintained between 37–38° C by a thermistor probe inserted subcutaneously and a thermostatically controlled infrared heat lamp. The electrophysiological techniques employed throughout are well standardized and adapted from those currently employed in clinical electroneuromyography.

Histopathological techniques

To study peripheral nerves morphologically, animals were killed with an overdose of pentobarbital. Peripheral nerve tissue was fixed with 0.1 M phosphate-buffered 4% glutaraldehyde solution either by transcardiac perfusion or in situ. The nerve was then cut into small blocks and postfixed with 2% OsO_4 in 0.1 M phosphate buffer, dehydrated with a series of graded ethanol, passed through propylene oxide and embedded in Epon 812. For light microscopy 1 μm sections were cut, mounted on slides and stained with thionin blue. For electron microscopy, ultrathin sections were doubly stained with uranyl acetate and lead citrate and examined with an Elmiskop CT 150 electron microscope.

For teasing single nerve fibres, the nerve was fixed in 0.1 M phosphate-buffered 4% glutaraldehyde, postfixed in 0.1 M phosphate-buffered OsO_4 and then placed in glycerin. Actual teasing was performed with the nerve in a pool of glycerin under a dissecting microscope.

Fluorescence microscopy

The unique autofluorescent properties of doxorubicin make it easily localizable in tissues (Bachur *et al.*, 1970; Egorin *et al.*, 1974; Arcamone, 1981*b*; Bigotte *et al.*, 1982*a*, *b*). Animals used for fluorescence microscopy were killed by an overdose of pentobarbital. Unfixed sciatic-tibial nerves were dissected rapidly and quick frozen in isopentane-liquid nitrogen. Sections of 6 and 8 μm were cut on a cryomicrotome, mounted on slides and cleared with a glycerin-water mixture (2 : 1). The specimens were examined with a Zeiss epifluorescence microscope (18F-2) with a BP455–490 filter. Viewed in this manner, doxorubicin has a characteristic orange-red fluorescence.

RESULTS

Electrophysiology

Microinjection of 30 μl of a 6.25 μg/ml concentration of doxorubicin (i.e., 0.19 μg) into rat sciatic nerve resulted in a reproducible sequence of electrophysiological changes. A delayed subacutely evolving demyelinative conduction block began at days 8–10 postinjection and became fully established by days 17–23. This conduction block remained for a further 20–25 days after which a slow but eventually complete return of conduction ensued. However, this conduction disturbance did not fully resolve until at least days 60–75 postinjection. Some nerves did not fully recover conduction until 90–100 days postinjection. Electrophysiological evidence of axonal degeneration was not apparent at this dosage (figs. 1A, 2).

Microinjection of 30 μl of a 12.5 μg/ml concentration of doxorubicin (i.e., 0.38 μg) produced a more profound subacute demyelinative conduction block with a similar time course as the 0.19 μg dose. There was minimal evidence of axonal degeneration at this dosage (fig. 1B).

Microinjection of 30 μl of a 100 μg/ml concentration of doxorubicin (i.e., 3 μg) produced once again a characteristic subacute demyelinative conduction block evolving at an even faster rate. However, at this dosage the focal demyelination was followed by a marked subacute motor axon degeneration, recorded as a progressive decline in the distal CMAP amplitude. The time course of these changes are shown in fig. 1C.

Neuropathological studies

Morphological studies showed a close correlation with the electrophysiological findings.

Fluorescence microscopy

In the tibial nerve sections from the site of injection, doxorubicin-specific fluorescence was present within the myelin of Schwann cells within 20 s of injection. By 1 min after injection doxorubicin-specific fluorescence was also noted in nuclei of Schwann cells (fig. 3). The location of this doxorubicin-specific fluorescence correlated with the subsequent distribution of Schwann cell degeneration and demyelination seen in peripheral nerve segments from other animals.

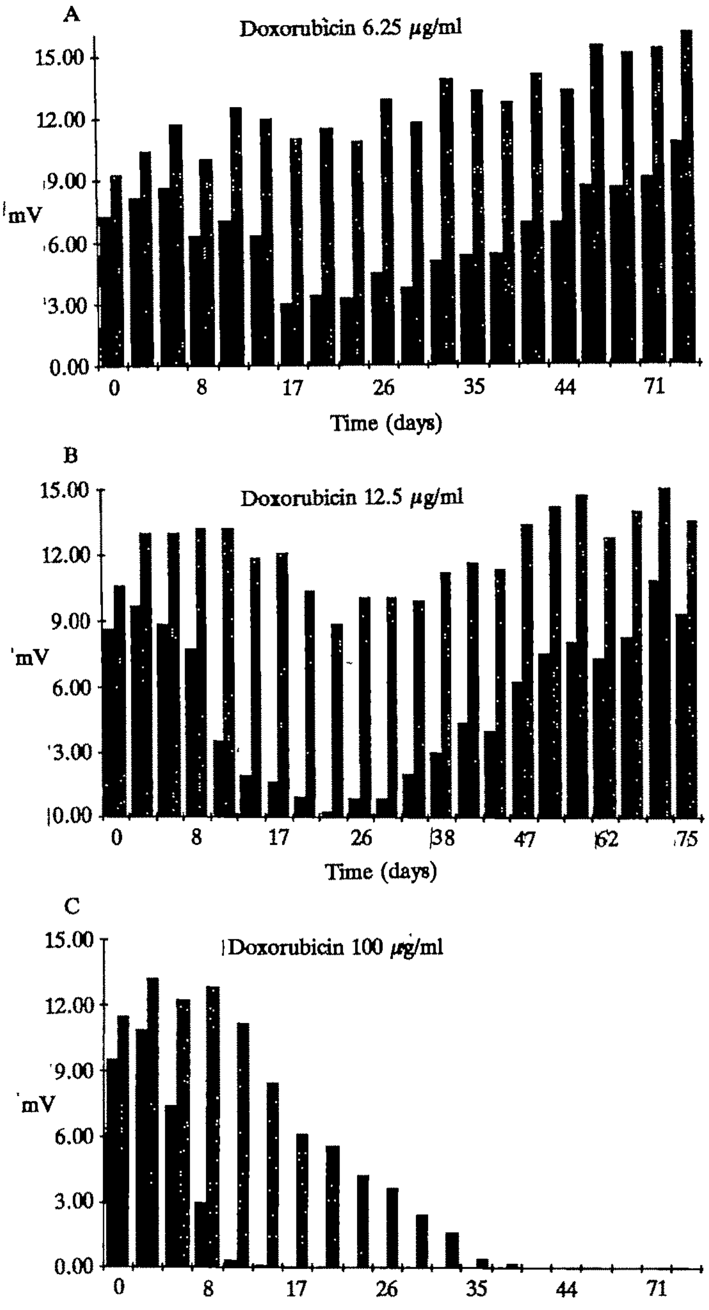

FIG. 1. Graph of serial mean compound muscle action potential (CMAP) amplitudes at various time points after injection of 30 μl of a 6.25 μg/ml concentration (i.e., total dose of 0.19 μg), a 12.5 μg/ml concentration (i.e., total dose of 0.38 μg) and a 100 μg/ml concentration (i.e , total dose of 3 μg) of doxorubicin. Solid bars represent proximal and stippled bars distal CMAP amplitudes. The decrease in the proximal CMAP amplitude (A, B, C) represents a focal conduction block secondary to demyelination and the decrease in the distal amplitude (C) represents motor axon degeneration (n = 20, 25 and 35 for A, B and C, respectively).

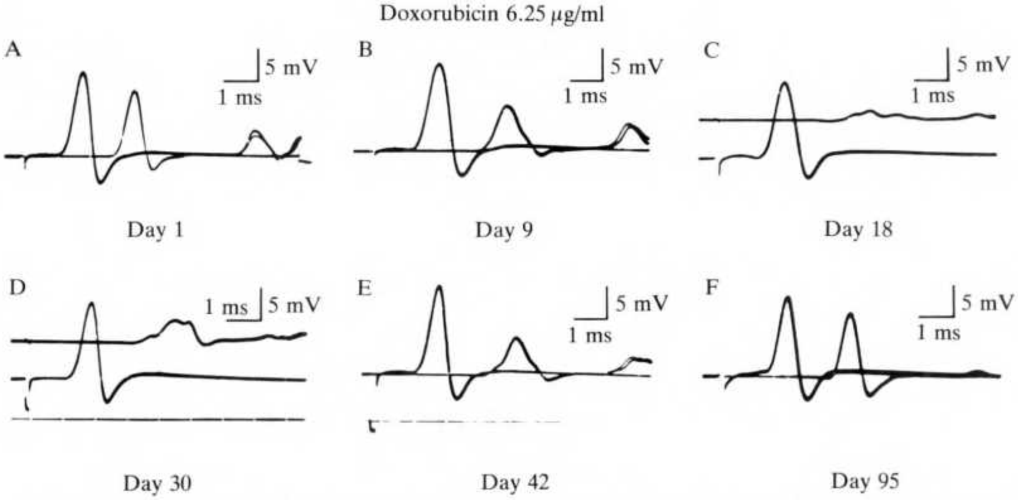

FIG. 2. A representative series of compound muscle action potentials (CMAPs) obtained after intraneural microinjection of 30 μl of a 6.25 μg/ml concentration of doxorubicin (i.e., total dose of 0.19 μg). The first peak in each series is the CMAP obtained by stimulation of the posterior tibial nerve distal to the injection site. The second peak in each series is the CMAP obtained by stimulation of the sciatic nerve proximal to the injection site. Notice the subacute evolution of a focal conduction block that becomes maximal by day 18. This is represented by the progressive decline in amplitude and temporal dispersion of the proximal CMAP. Thereafter there is a slow but eventually complete (day 95) return of conduction. The distal CMAP amplitude remains unchanged, and thus significant axonal degeneration is not apparent at this dosage.

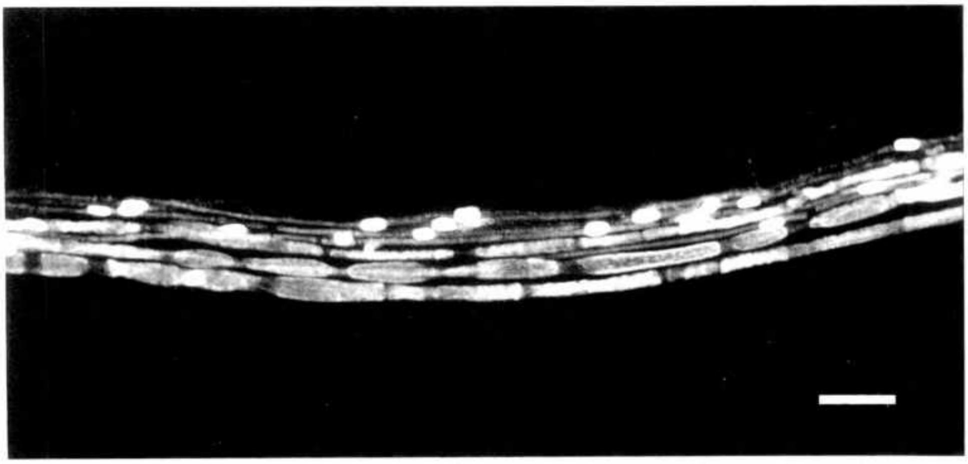

FIG. 3. Fluorescent photomicrograph of a teased fibre preparation of the tibial nerve 1 min after injection of 0.38 μg of doxorubicin. Doxorubicin-specific fluorescence is already present in the nuclei of Schwann cells and in the myelin sheaths of axons. Bar = 40 μm.

Light microscopy

The Schwann cell and myelin changes were qualitatively similar at all doses of doxorubicin; therefore only the changes induced by 0.38 μg of doxorubicin will be described in detail. The earliest abnormality at the site of injection consisted of the separation of myelin lamellae by the process of intramyelinic oedema. This was first noticed at days 7–8 postinjection. By 10 days postinjection paranodal demyelination was seen involving several fibres. At 12–13 days, extensive segmental demyelination was present. Myelin debris surrounded denuded axons and many macrophages filled with myelin debris were seen. The demyelination became progressively more extensive so that by days 22–27 postinjection approximately 75–80% of all the fibres in a cross-section were denuded by myelin. Longitudinal sections at this time showed very long segments of demyelinated axons (fig. 4).

Teased fibre preparations at this and at slightly later times showed long segments of demyelinated fibres (fig. 5). Early remyelination was first noted at 28–33 days postinjection. This consisted of axons with inappropriately thin (and thus presumably new) myelin sheaths. Remyelination progressed until as long as 90–100 days after injection (fig. 6).

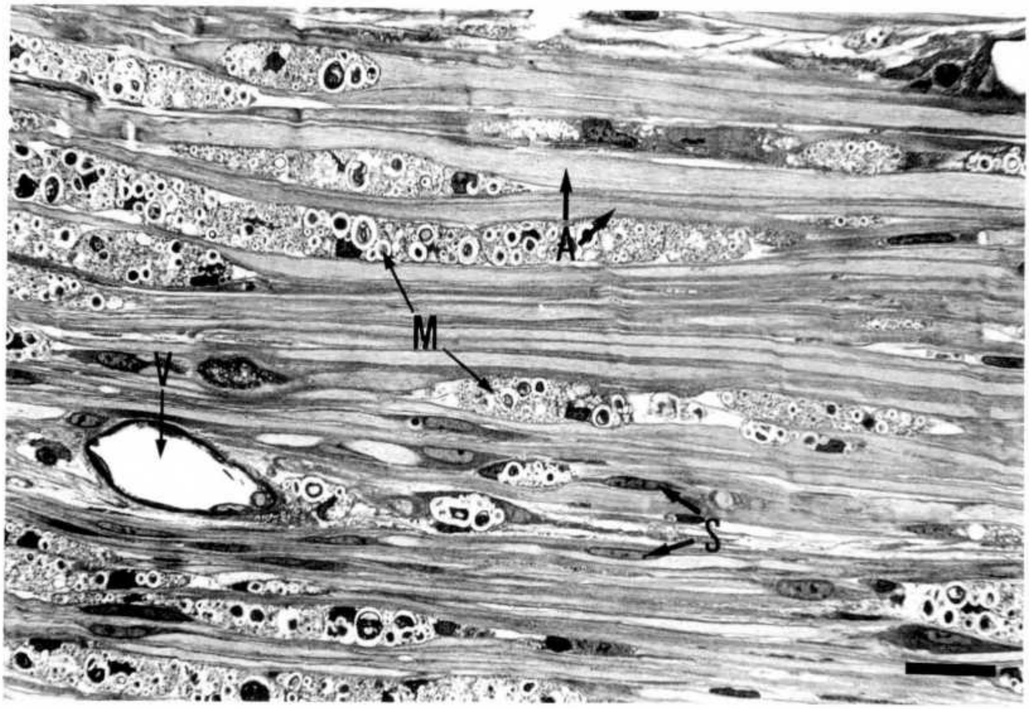

FIG. 4. Longitudinal section of tibial nerve 26 days after injection of 0.38 μg of doxorubicin. Note long segments of demyelinated axons (A). M, macrophage filled with myelin debris; V, endoneurial blood vessel; S, nucleus of Schwann cell. Bar = 20 μm.

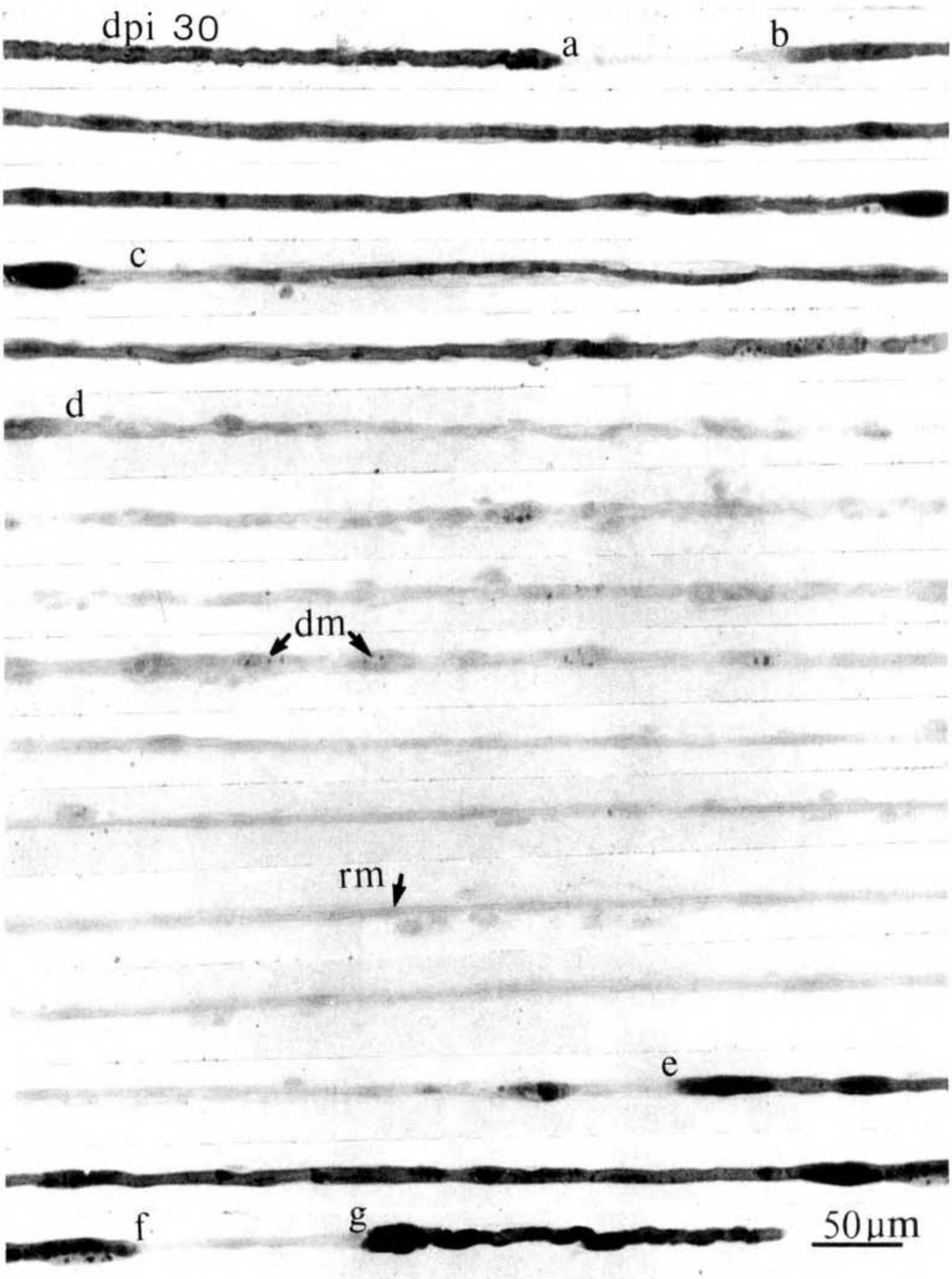

FIG. 5. Teased fibre preparation at day 30 after injection of 0.38 μg of doxorubicin. Note the long segments of demyelination (a–b, c, d–e, f–g). Myelin debris (dm) and an early remyelinating (rm) section are also noted. Bar = 50 μm.

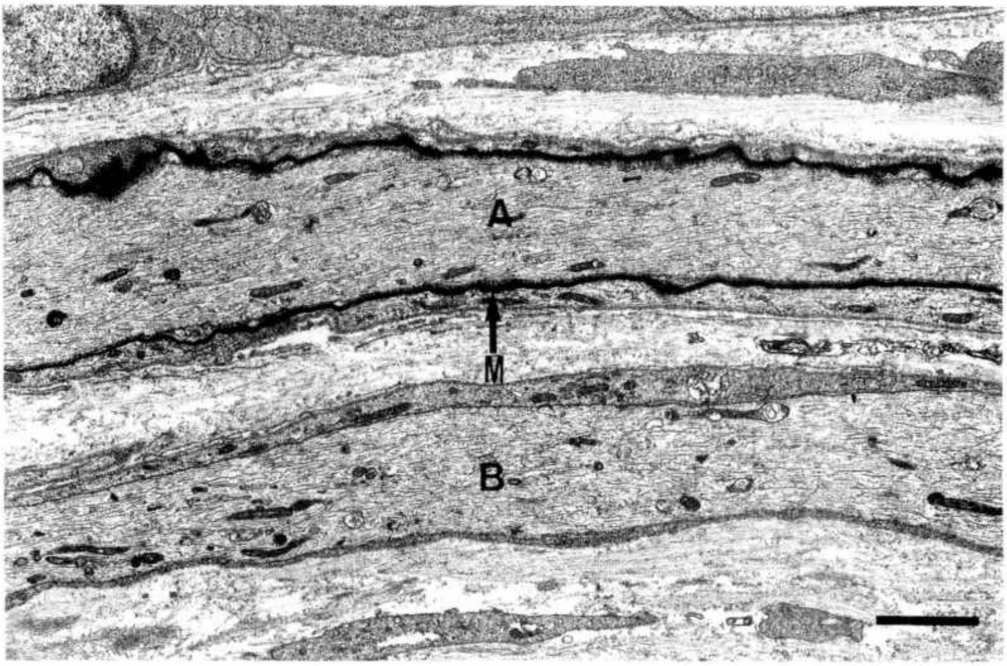

FIG. 6. Early remyelination (M) of an axon (A) at day 43 after injection of 0.38 μg of doxorubicin. The axon indicated by B is still demyelinated. Bar = 2 μm.

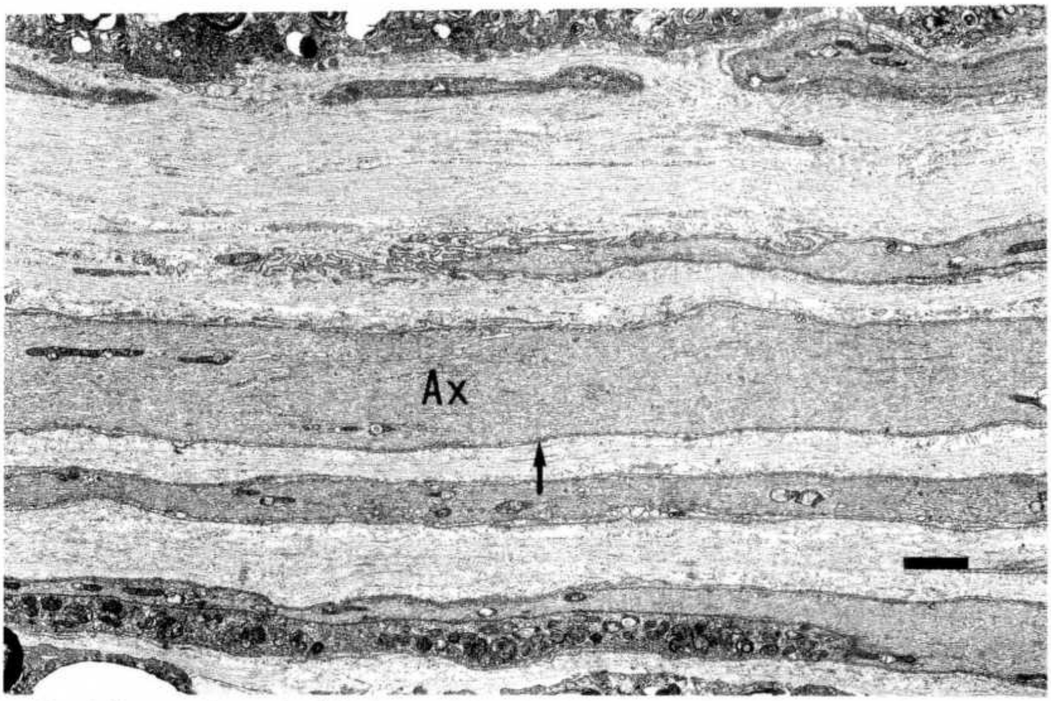

FIG. 7. Electron micrograph of tibial nerve 27 days after injection of 0.19 μg of doxorubicin. Axons (Ax) are surrounded only by a Schwann cell basal lamina (arrow) without Schwann cell cytoplasm or myelin. Bar = 1.3 μm.

Tibial nerve segments 1.5 cm distal to the site of injection were examined at days 46 and 100 postinjection. There was no significant axonal degeneration in nerves injected with 0.19 μg of doxorubicin, and minimal axonal degeneration (< 5%) in nerves injected with 0.38 μg of doxorubicin; however, there was considerable (apparently 40%) degeneration of axons in nerves injected with 3 μg of doxorubicin.

Electron microscopy

Ultrastructural findings paralleled the light microscopic findings. At the peak of demyelination, almost all the fibres were totally demyelinated and surrounded by macrophages filled with myelin debris. Many fibres were surrounded only by a basal lamina without Schwann cell cytoplasm (fig. 7).

DISCUSSION

Relatively few agents are regarded as Schwann cell toxins. Tellurium produces transient demyelination when fed orally to weanling and adult rats (Lampert *et al.*, 1970; Lampert and Garrett, 1971; Duckett *et al.*, 1979; Said and Duckett, 1981). Systemic administration of lead to adult rats causes a variable degree of primary segmental demyelination that is dependent upon the schedule of chronic low dose administration (Lampert and Schochet, 1968; Windebank and Dyck, 1984). The majority of evidence suggests that this demyelination is secondary to Schwann cell damage (Dyck *et al.*, 1977; Ohnishi *et al.*, 1977; Myers *et al.*, 1980; Powell *et al.*, 1982). Diphtheria toxin, which has been used extensively for morphological and electrophysiological studies of demyelinated nerve fibres (Sears and Bostock, 1981; McDonald and Kocen, 1984), has been shown to inhibit protein synthesis by specifically inactivating eEF2, the elongation factor for translational movements of ribosomes along messenger RNA (Pappenheimer and Gill, 1973). Demyelination is thus due to inhibition of protein synthesis in Schwann cells. However, both lead and diphtheria toxin result in complicated patterns of simultaneous degenerative and regenerative changes in Schwann cells so that it has proved difficult to trace the mechanisms by which Schwann cell damage leads to demyelination.

Tunicamycin has been shown in vitro to inhibit Schwann cell glycation of glycoproteins in peripheral myelin (Smith and Sternberger, 1982). When microinjected into the endoneurial space of rat sciatic nerve, tunicamycin produces a delayed and pronounced vesicular demyelination that begins after 3 to 8 days. This is followed by early Schwann cell proliferation and remyelination beginning at about 15 days. These findings have been interpreted as indicating that inhibition of metabolic pathways within the Schwann cell were relatively selective for myelin maintenance resulting in demyelination without affecting the ability of the Schwann cell to proliferate and remyelinate (Sumner *et al.*, 1983).

Antimitotic agents have been little explored as Schwann cell toxins. Hall and Gregson (1974) noted that after demyelination of peripheral nerve produced by injection of lysophosphatidyl choline, mitomycin-C caused a marked delay in remyelination. This was thought to be secondary to inhibition of Schwann cell replication by the mitomycin-C. They did not, however, report that mitomycin-C alone can produce demyelination. Pollard *et al.* (1983) reported that the intraneural injection of mitomycin-C results in a profound delayed demyelination of nerve. Morphological studies were consistent with a toxic disintegration of Schwann cells.

Our current study clearly demonstrates that another antimitotic agent, doxorubicin, can produce profound demyelination of peripheral nerve when introduced into the endoneurium. The important characteristics of this demyelination are its delayed onset, subacute evolution and prolonged recovery. For instance, with the injection of 0.19 μg of doxorubicin, the demyelinative conduction block does not start until days 8–10 postinjection and is not fully established until days 17–23. This conduction block (the electrophysiological hallmark of a focal demyelinative lesion) (McDonald, 1980) remains for a further 20–25 days, after which a slow but eventually complete return of conduction ensues. However, the remyelination process is not electrophysiologically complete until at least days 60–75 postinjection. With higher quantities of doxorubicin the time course of the development of demyelination is slightly accelerated but still conforms to a subacutely evolving process. Ultrastructurally, axons were denuded of myelin for considerable distances (i.e., more than one internode) and although these axons were still invested within Schwann cell basal lamina, Schwann cells themselves could not be seen. These findings are most consistent with selective intoxication and death of myelin-associated Schwann cells producing primary segmental demyelination.

Since the mode of action of doxorubicin is intercalation between base pairs of DNA with inhibition of messenger RNA synthesis (Pigram *et al.*, 1972; Di Marco, 1975; Arcamone, 1981*a*), Schwann cell degeneration must occur as a consequence of this effect. Although Schwann cell multiplication in rat peripheral nerve is intense neonatally, this mitotic activity virtually ceases in adult animals (Asbury, 1967; Perkins *et al.*, 1981); therefore, the initial effect of doxorubicin on mature Schwann cells presumably occurs as a result of an ultimate disruption of cellular protein synthesis. With disruption of Schwann cell metabolic processes, myelin production and maintenance are affected, and finally other vital Schwann cell activities are disrupted culminating in cell death. On the other hand, the marked delay in remyelination may indeed result from the antimitotic effect of doxorubicin. The remyelination in this setting presumably occurs as unaffected Schwann cells (from the uninjected segments of nerve) migrate into the demyelinated segment, proliferate and reinvest naked axons. Inhibition of this Schwann cell proliferation by doxorubicin could account for the profoundly delayed remyelination. A similar conclusion was reached by Hall and Gregson (1974) in their experiments with mitomycin-C.

At the lowest quantity of doxorubicin injected (i.e., 0.19 μg), significant axonal degeneration was not apparent. This conclusion is based on the findings of persistently normal amplitude CMAPs elicited by stimulation of nerve distal to the site of injection and the absence of significant axonal degeneration in morphological specimens distal to the site of injection. Thus at this dosage a relatively selective effect of Schwann cells occurs, producing a purely demyelinative lesion.

As the dosage of doxorubicin is increased to 0.38 μg, a minor degree of axonal degeneration appears, and at doses greater than 3 μg, a profound delayed motor axon degeneration occurs. Electrophysiologically this is signalled by a progressive decline in the distal CMAP. Neuropathological studies indicate a concomitant motor neuron (anterior horn cell)-axonal degeneration. This motor neuron degeneration is discussed in the following communication (England *et al.*, 1988).

With respect to Schwann cells, it is clear that doxorubicin in minute quantities can cause their selective degeneration. Thus this agent can be used to produce an in vivo model of primary segmental demyelination that is a result of a toxic Schwannopathy. This local cytotoxic effect upon Schwann cells provides an opportunity to study the in vivo interrelationships among Schwann cell, myelin sheath and axon. More intensive study of such toxic Schwannopathies and the demyelination thus produced may provide further clues concerning the basic biological mechanisms that are involved in the pathogenesis of human demyelinative neuropathies. Moreover, the ability to kill Schwann cells focally and selectively and to inhibit their proliferation offers the possibility of studying the role of these cells in other peripheral nerve processes such as wallerian degeneration and axonal regeneration.

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LETHAL RETROGRADE AXOPLASMIC TRANSPORT OF DOXORUBICIN (ADRIAMYCIN) TO MOTOR NEURONS

A TOXIC MOTOR NEURONOPATHY

by JOHN D. ENGLAND, ARTHUR K. ASBURY, EDWARD K. RHEE *and*
AUSTIN J. SUMNER

(*From the Department of Neurology, Hospital of the University of Pennsylvania,
Philadelphia, USA*)

SUMMARY

Doxorubicin (Adriamycin) is an autofluorescent anthracycline antibiotic that acts as a DNA intercalator. Following intraneural microinjection of 3 μg of doxorubicin into rat tibial nerve, fluorescence microscopy indicated that it is transported retrogradely to anterior horn cell bodies. Subsequently, these motor neurons underwent a progressive subacute degeneration that occurred over a period of 35–39 days. Combined electrophysiological and neuropathological methods indicated that dorsal root ganglion cells were relatively unaffected by this dose of toxin. The selective motor neuron degeneration produced by this agent raises the possibility that abnormalities of nucleic acid metabolism may be involved in the aetiology of motor neuron diseases.

INTRODUCTION

Doxorubicin is an anthracycline antineoplastic antibiotic that acts at the cell nucleus by intercalating between the base pairs of DNA, thus inhibiting DNA-directed messenger RNA synthesis (Pigram *et al.*, 1972; Di Marco, 1975). The intraneural injection of this substance causes focal Schwann cell degeneration and consequent demyelination (England *et al.*, 1988). With quantities of doxorubicin larger than those required to intoxicate Schwann cells selectively, this agent can undergo preferential retrograde axoplasmic transport to motor neuron nuclei, resulting in a relatively selective motor neuron degeneration. This experimental motor neuronopathy may therefore provide a model of motor neuron disease. The electrophysiological and neuropathological effects of this toxin upon motor neurons form the basis of this report.

Correspondence to: Dr Austin J Sumner, Department of Neurology, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA.

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METHODS

Intraneural injection

Male Wistar rats (350–400 g) were used in these experiments. Doxorubicin (Adriamycin) was diluted in normal saline to a concentration of 100 μg/ml, and 30 μl of this solution (i.e., 3 μg Adriamycin) were injected into the subperineurial region of the single fascicle of the tibial nerve. The details of this procedure have been described previously (England *et al.*, 1988). For each animal only one side was injected so that the contralateral (uninjected) side could serve as a control.

Electrophysiological studies

Compound muscle action potentials (CMAPs) were recorded from the intrinsic foot muscles after stimulation of the sciatic nerve at the sciatic notch (proximal stimulation) and the tibial nerve at the ankle (distal stimulation). The details of this procedure have also been described previously (England *et al.*, 1988).

In 6 animals, nerve action potentials (NAPs) were recorded from the tibial nerve and its contributing dorsal and ventral roots (L4, L5 and L6) after stimulation of the tibial nerve at the ankle. For these studies the entire sciatic-tibial nerve as well as the L4, L5 and L6 dorsal and ventral roots were exposed surgically, dissected and isolated. When the surgical field was clean and free of bleeding points, the nervous tissue was covered in a paraffin bath continuously bubbled with carbogen. The temperature of the bath was maintained between 37–38° C by a thermostatically controlled heating device. A pair of platinum tipped steel electrodes (Grass E2) placed alongside the tibial nerve at the ankle were used for stimulation. Square-wave pulses of current 0.1 ms in duration were delivered at a rate of 1 Hz. DISA near-nerve recording electrodes were placed in a bipolar arrangement under the tibial nerve at the popliteal fossa and under the appropriate dorsal and ventral roots (L4, L5 and L6). These potentials were recorded on a TECA TD20 and averaged electronically using a frequency band of 20 Hz–2 kHz. These nerve conduction study arrangements are summarized in fig. 1.

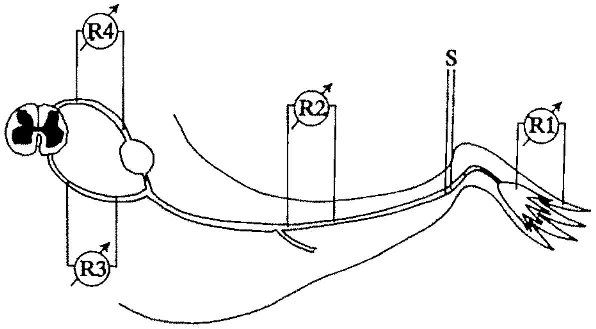

FIG. 1 The tibial nerve is stimulated at the ankle (S). Compound muscle action potentials (CMAPs) are recorded from intrinsic foot muscles (R1); nerve action potentials (NAPs) are recorded direct from proximal tibial nerve (R2), ventral roots (R3), and dorsal roots (R4).

Histopathological techniques

Animals for histopathological study were killed with an overdose of pentobarbital. Following transcardiac fixation with 0.1 M phosphate-buffered 4% glutaraldehyde, lower lumbosacral spinal cord and bilateral L4–L6 dorsal root ganglia were dissected free. Sciatic and tibial nerves on the side of injection were sometimes also obtained. The spinal cord was cut into transverse blocks from both rostral and caudal lumbar enlargement. Peripheral nerve was cut into small blocks from nerve segments proximal, distal and at the site of injection. Dorsal root ganglia were fixed in toto. All tissue was then postfixed with 2% OsO_4 in a 0.1 M phosphate buffer, dehydrated in a series of graded ethanol, passed through propylene oxide and embedded in Epon 812. For light microscopy 1 μm sections were cut, mounted on slides, stained with methylene blue-azure II and counterstained with basic fuchsin.

Fluorescence microscopy

Since doxorubicin has unique autofluorescence properties (Bachur *et al.*, 1970; Egorin *et al.*, 1974; Arcamone, 1981; Bigotte *et al.*, 1982*a*, *b*), it is easily localized in tissues using fluorescence microscopy. Animals used for fluorescence studies were killed by an overdose of pentobarbital. Unfixed caudal lumbar spinal cord segments (L4, L5, L6) and their dorsal root ganglia were rapidly dissected and quick frozen in isopentane-liquid nitrogen. Sections (8 μm) were cut in a cryomicrotome, mounted on slides, and cleared with a glycerin–water mixture (2:1). The specimens were examined with a Zeiss epifluorescence microscope (18F-2) with a BP 455–490 filter. Viewed in this manner doxorubicin has a characteristic orange-red fluorescence.

RESULTS

Location of doxorubicin-specific fluorescence

Doxorubicin-specific fluorescence was first detected in anterior horn cells of the caudal lumbar cord (L4–6) at 48 h following injection. At this time nuclei were just beginning to fluoresce, and the number of fluorescent nuclei was limited to a few per section. With time the fluorescence increased in intensity until it reached a maximum at 4–5 days following injection. During this period strikingly intense and distinct fluorescence was seen in the nuclei of many anterior horn cells in the L4–6 segments. Additionally, intense fluorescence was seen in the nuclei of satellite cells situated around the fluorescent anterior horn cells (fig. 2). After 5 days the fluorescence gradually faded but was still present in many anterior horn cells and surrounding satellite cells at 8–10 days following injection. At these times, however,

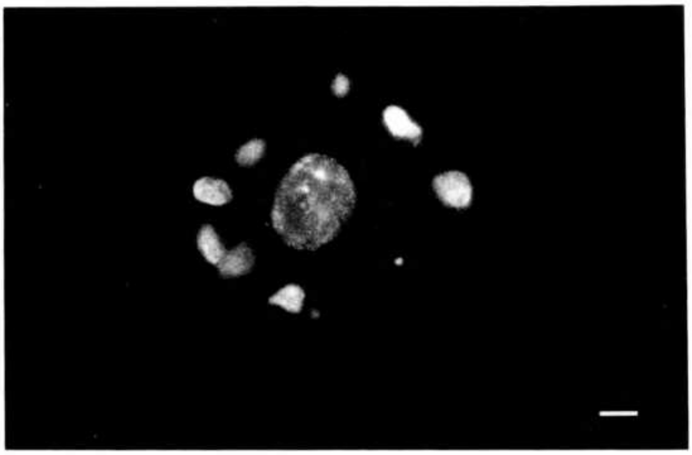

FIG. 2. Fluorescent photomicrograph showing doxorubicin-specific fluorescence in an anterior horn cell nucleus and in nuclei of surrounding satellite cells at 4 days after injection of 3 μg of doxorubicin. Bar = 10 μm.

most cells showed fluorescence only at the nuclear periphery, giving a ring-like appearance in contrast to the diffuse nuclear fluorescence seen earlier. The doxorubicin-specific fluorescence was always well localized to the ventral horn ipsilateral to the injection; this fluorescence was not seen in the contralateral ventral horn.

At the same times, doxorubicin-specific fluorescence was rarely detectable, and then only weakly, in dorsal root ganglion cells from L4-6 segments.

Effects on Schwann cells

Microinjection of 3 μg of doxorubicin into rat peripheral nerve resulted first in a delayed subacutely evolving demyelinative conduction block secondary to Schwann cell degeneration. This Schwann cell degeneration has already been reported (England *et al.*, 1988) but requires to be mentioned here because an awareness of this prolonged focal demyelination is necessary for a complete understanding of the electrophysiological studies that follow.

Effects on motor neurons

Electrophysiological studies were performed on 35 animals after intraneural injection of 3 μg of doxorubicin. Focal demyelination was followed by a subacute motor neuron-axon degeneration. Electrophysiologically this neuron-axon degeneration was recorded as a progressive decline in the distal CMAP. Distal motor axon failure occurred in some axons as early as days 13–14 postinjection, whereas others did not fail until as late as days 35–39 days postinjection (fig. 3).

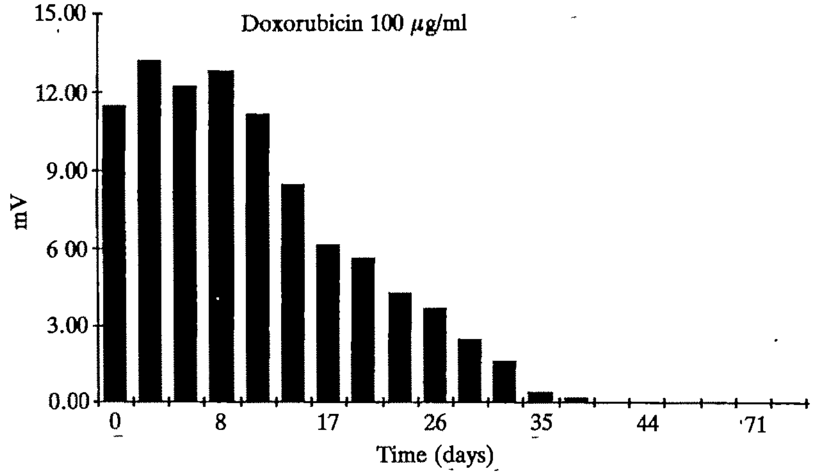

FIG. 3. Graph of serial mean distal compound muscle action potential (CMAP) amplitudes at various time points after injection of 30 μl of a 100 μg/ml concentration (i.e., total dose of 3 μg) of doxorubicin. The statistically significant decline in amplitude starts at days 13–14 (*P* at 0.01 using the Tukey-Kramer multiple comparison procedure) (Ott, 1984; Kramer, 1956, 1957). This decline in amplitude is not complete until day 39 (n = 35). The SD is less than 2.9 for all of these means. Correlative neuropathological observations show a concomitant anterior horn cell degeneration.

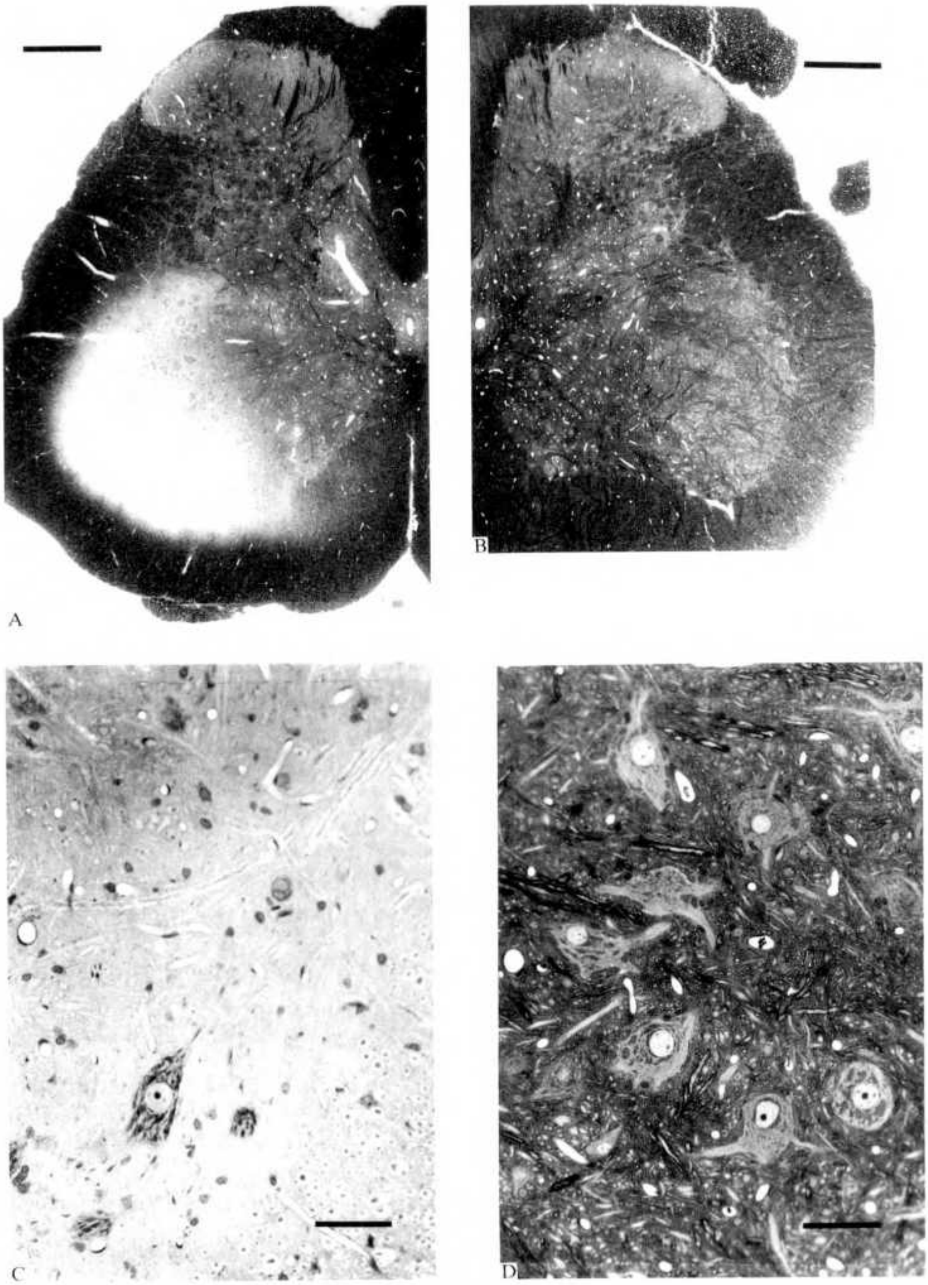

FIG. 4. Comparable photomicrographs of caudal lumbar anterior horns of the side ipsilateral to injection of 3 μg of doxorubicin (A, C) and the contralateral side (B, D). C and D are higher magnification views of the anterior horns of A and B respectively. Note the marked devastation of neuropil and decrease in the number of anterior horn cells in A and C as contrasted with the normal appearance in B and D. Both specimens 40 days postinjection. A, B, bar = 353 μm; C, D, bar = 62.5 μm.

Neuropathological studies showed that the motor axon degeneration was accompanied by progressive anterior horn cell degeneration (fig. 4). At a time when electrophysiological studies indicated complete degeneration of all motor axons previously innervating the intrinsic foot muscles (days 35–39 postinjection), spinal cords were obtained from 16 animals; 1 μm sections of L4–6 cord segments were examined in detail by light microscopy. Anterior horn cell counts were performed by counting the number of anterior horn cells with visible nucleoli in × 160 microscopic fields and comparing numbers ipsilateral and contralateral to the side of injection. These counts showed a highly significant decrease ($P < 0.001$, paired Student's t test) in the number of anterior horn cells ipsilateral to the side of injection ($\bar{X} = 8 \pm$ SD 1.03) as compared with the contralateral control side ($\bar{X} = 17 \pm$ SD 1.51).

Dissociation between the susceptibility of motor neurons versus dorsal root ganglion cells

Simultaneous study of primary sensory neuron-axons showed that, at least in the minute amounts of doxorubicin that we have used, there is a dissociation in the susceptibility of primary sensory neurons as compared with motor neurons. Ten animals were studied by recording nerve action potentials (NAPs) from the tibial nerve and concomitantly recording compound muscle action potentials (CMAPs) from intrinsic foot muscles after stimulation of the tibial nerve at the ankle. During the time when motor axons of the tibial nerve underwent subacute

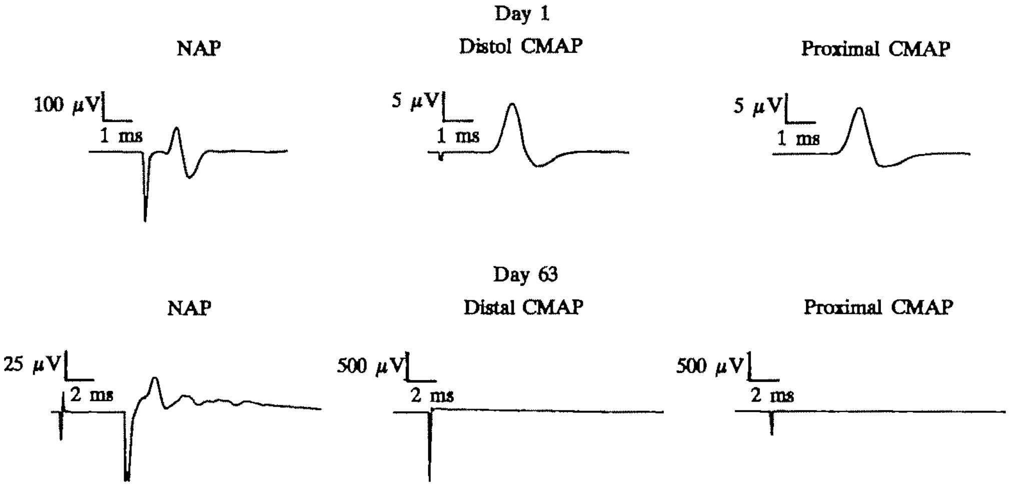

FIG. 5. A representative sequence of tibial nerve action potentials (NAPs) and compound muscle action potentials (CMAPs) recorded after injection of 3 μg in 30 μl of doxorubicin into the tibial nerve. Notice that at day 63, the CMAPs are unelicitable, whereas the tibial NAP is well preserved (although, as expected, decreased in amplitude from day 1). At day 63, the tail of the tibial NAP also shows some temporal dispersion, indicating conduction through a segment of residual demyelination.

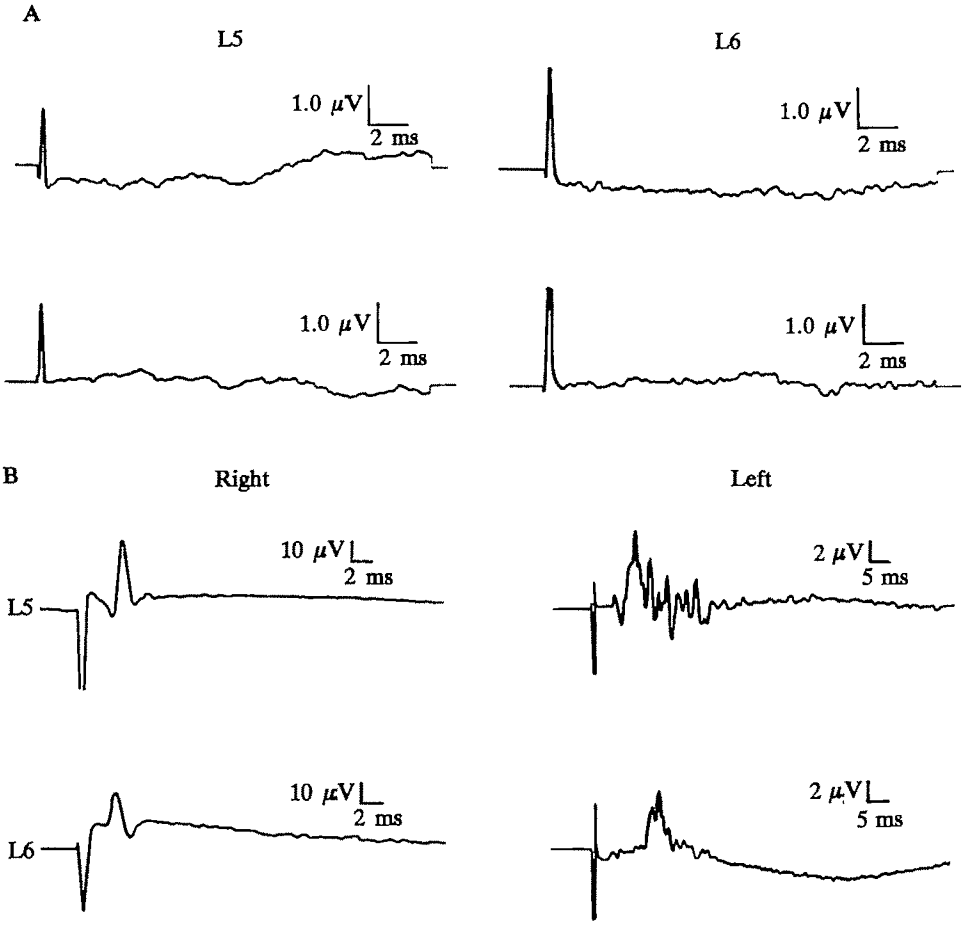

FIG. 6. A, L5 and L6 ventral root recordings ipsilateral to the injection at day 63 postinjection. Note that there is no reproducible response. B, L5 and L6 dorsal root recordings contralateral (*right*) and ipsilateral (*left*) to the injection at day 63 postinjection (same animal as in A). Note that dorsal root potentials on the left side are definitely present although decreased in amplitude and temporally dispersed in comparison to those on the right, indicating conduction through a segment of residual demyelination.

degeneration with a fall of the distal CMAP to zero over a period of 35–39 days, the NAPs recorded directly from this nerve remained well preserved (fig. 5).

Concomitant direct in vivo ventral and dorsal root studies were performed on 6 of these animals. Following distal tibial nerve stimulation, direct recordings were made from L4, L5 and L6 roots. At a time when distal CMAPs were unelicitable, ventral root potentials were also unelicitable (fig. 6A); dorsal root potentials, however, were preserved (fig. 6B). Thus at a time when there was complete degeneration of motor axons directed to intrinsic foot muscles, there was well preserved function of sensory axons representative of the same spinal segments.

Lower lumbar (L4–6) spinal cord segments with accompanying dorsal root

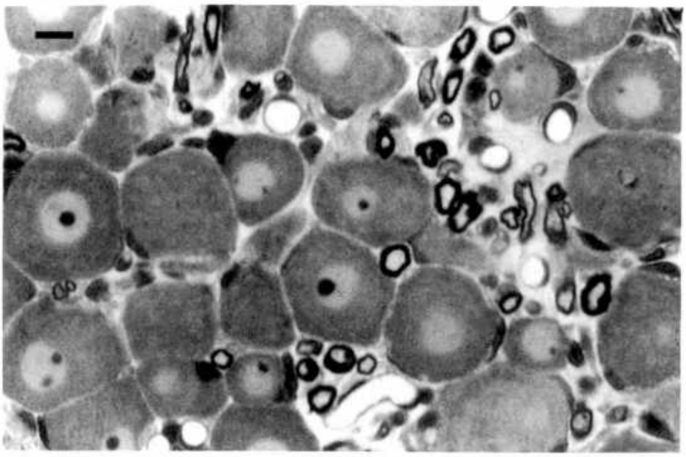

FIG. 7. L6 dorsal root ganglion ipsilateral to injection at day 46 postinjection. Note that there is no identifiable structural abnormality. Bar = 15.8 μm.

ganglia were examined in the 6 animals also studied electrophysiologically. Specimens were taken at days 38, 45, 55, 63, 70 and 87 postinjection. These specimens showed significant anterior horn cell loss on the side ipsilateral to injection (as described in the previous section) with no identifiable structural alteration of dorsal root ganglia from the same spinal segments and ipsilateral to the side of injection (fig. 7). Further detailed examination of dorsal root ganglia in 10 additional animals similarly injected (but whose NAPs were not recorded) confirmed these neuropathological findings. Hence the combined electrophysiological and morphological studies indicated a significant difference in the vulnerability of primary motor neurons as compared with primary sensory neurons.

DISCUSSION

Previous investigators have demonstrated motor neuron degeneration following both intramuscular and intraneural injection of doxorubicin. Bigotte and Olsson (1982, 1983) showed degeneration of mouse hypoglossal motor neurons following retrograde axonal transport of doxorubicin from the tongue. Yamamoto *et al.* (1984) demonstrated anterior horn cell and dorsal root ganglion cell degeneration after subepineurial sciatic nerve injection of 70 μg of doxorubicin.

By injecting doxorubicin in quantities much smaller than those used by these investigators and employing correlated electrophysiological and neuropathological methods, we have further refined and elaborated on these initial observations. Intraneural microinjection of 3 μg of doxorubicin is followed by retrograde axoplasmic transport of this substance to motor neuron nuclei, causing these cells to degenerate. Further, at least in the minute dosages that we have used, the motor neurons are significantly more vulnerable than sensory neurons (dorsal root ganglion cells). Since doxorubicin-specific fluorescence was rarely detected in dorsal root ganglion cells at a time when motor neurons showed discrete and vivid fluorescence, this difference in susceptibility most likely reflects, at least in part, a difference in toxin uptake and/or retrograde axoplasmic transport between these two cell types. In contrast, intravenous injection of large doses of doxorubicin produces a selective dorsal root ganglion cell degeneration (Cho, 1977; Cho *et al.*, 1979; Bronson *et al.*, 1982; Eddy and Nathaniel, 1982). This is most probably a result of ready access of the toxin to these cells, which lack a blood-nerve permeability barrier. Yamamoto *et al.* (1984) also found dorsal root ganglion cell degeneration following injection of doxorubicin into rat sciatic nerve; however, they used 70 μg of doxorubicin and crushed the injection site. These studies demonstrate that dorsal root ganglion cells can be intoxicated if the quantity of doxorubicin that reaches their nuclei is great enough. Our demonstration of a differential intoxication of motor neurons versus dorsal root ganglion cells is not in conflict with the observations of Yamamoto *et al.* (1984) as we used much lower doses (3 μg) of doxorubicin administered intraneurally. Thus under appropriate circumstances, doxorubicin can produce a selective motor neuron degeneration. As the mechanism of action of doxorubicin is DNA intercalation, its cytotoxic effect on motor neurons must be a result of impaired RNA synthesis and eventually impaired protein synthesis. This effect is consistent with the delayed and progressive motor neuron degeneration that occurs after the incorporation of doxorubicin into the motor neuron nucleus.

The action of doxorubicin on motor neurons may be relevant to an understanding of the pathogenesis of human motor neuron diseases. There is already a considerable body of evidence indicating major abnormalities of neuronal nucleic acid metabolism in both human and animal motor neuron diseases. Mann and Yates (1974) first reported reduced levels of cytoplasmic RNA in anterior horn cells that occurred in parallel with decreased nuclear and nucleolar volumes. They postulated that this was related to a primary heterochromatization of nuclear DNA with resultant impaired synthesis of messenger RNA (Mann and Yates, 1974). Davidson *et al.* (1981, Davidson and Hartmann (1981*a*)) and Hartmann and Davidson (1982) likewise demonstrated a 31–42% reduction in neuronal RNA content in lumbar and cervical cord motor neurons, but not in nucleus dorsalis neurons. This reduction in RNA content was independent of disease duration and extent of cell loss. The base composition of RNA from the larger motor neurons in patients with amyotrophic lateral sclerosis (ALS) was also abnormal with a lower percentage of adenine and a lower ratio of adenine to uridylic acid (Davidson and Hartmann, 1981*b*). Additionally, cultured fibroblasts from ALS patients appear to show an impaired DNA repair capacity following exposure to the alkylating agent methyl methanesulfonate (Tandan and Bradley, 1985). There is also some preliminary evidence that ALS cells may be exquisitely sensitive to ionizing radiation (Kidson *et al.*, 1983).

In the wobbler mouse, a mutant that suffers from a recessively inherited degeneration of motor neurons in the spinal cord and brainstem, Murakami *et al.*

(1980, 1981) have found several abnormalities in spinal motor neurons that suggest abnormal RNA metabolism. Specifically, they found decreased RNA content, decreased synthesis of nuclear and nucleolar RNA, decreased protein synthesis, and increased protein turnover.

Because of these and other observations, Bradley and Krasin (1982*a*, *b*) have advanced the hypothesis that the primary abnormality in ALS is the accumulation of abnormal DNA, which results from abnormal or deficient DNA repair mechanisms. Transcription of messenger RNA and thus protein synthesis would be disrupted, eventually causing motor neuron degeneration. Our study provides some support for this hypothesis, for it shows that an agent that directly interferes with nucleic acid metabolism can cause a selective motor neuron degeneration. Such models of toxic derangement of nucleic acid metabolism may indeed provide useful insights into the pathogenesis of the human motor neuron diseases.

Recently there has been a resurgence of interest in the possibility of toxic causes of degenerative disease, including ALS. ALS previously occurred in high incidence (annual incidence of 50/100,000 in the 1950s) among the Chamorro people of Guam; however, over the past 30 years, the incidence of this disease has been steadily declining and present rates (annual incidence of $< 5/100{,}000$) are only slightly greater than those in the continental United States. This rapid near disappearance of this high incidence focus strongly suggests that environmental factors were aetiological (Garruto *et al.*, 1985). After noting that cycad, *Cycas circinalis*, was an important source of food for the Chamorros, Kurland (1972) had previously suggested that a toxin from this plant could cause ALS. Recently, Spencer *et al.* (1986) have shown that L-BMAA (beta-N-methylamino-L-alanine) from the cycad seed can cause motor neuron degeneration. Daily feeding of macaques with BMAA causes Betz cell and anterior horn cell degeneration. Thus neurotoxic agents deserve serious consideration as potential causative agents of ALS on Guam and possibly also elsewhere.

Another intriguing aspect of the doxorubicin model relates to the cell to cell transfer of this toxin. Our fluorescent studies suggest that doxorubicin moves from anterior horn cells to surrounding satellite cells. The marked devastation of ventral horns in our chronic spinal cord specimens also implies that not only anterior horn cells but also surrounding cellular elements have degenerated. Based on these observations, the transfer of doxorubicin to central motor neurons making monosynaptic contact with anterior horn cells is a distinct possibility. If such interneuronal transfer of toxicity occurs, it would further validate the usefulness of doxorubicin in producing a model of motor neuron disease and could provide new insight into the action of other nervous system toxins.

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SERIAL GADOLINIUM ENHANCED MAGNETIC RESONANCE IMAGING IN MULTIPLE SCLEROSIS

by D. H. MILLER, P. RUDGE, G. JOHNSON, B. E. KENDALL,
D. G. MACMANUS, I. F. MOSELEY, D. BARNES *and* W. I. MCDONALD

(*From the Institute of Neurology and National Hospitals for Nervous Diseases,
Queen Square, London*)

SUMMARY

Serial gadolinium-DTPA (Gd-DTPA) enhanced magnetic resonance imaging (MRI) was performed in 9 patients with multiple sclerosis (MS). On the first scan enhancing lesions were seen in 7 patients, all of whom were in acute relapse. Most enhancing lesions were asymptomatic. On the second scan (3 to 5 weeks later), persisting enhancement was seen in only 12/54 lesions which enhanced on the first scan. No lesion showed persisting enhancement on the third scan (after 6 months).

Enhancement occurred in all 12 new lesion areas seen on the unenhanced second scan and in 8 of 15 new lesions seen on the third scan. Enhancement was also seen in 4 older lesions which had been nonenhancing on earlier scans. Relaxation time measurements demonstrated a high water content in some nonenhancing lesions, which could be due to a subtle blood-brain barrier disturbance not detected with Gd-DTPA.

The results show that blood-brain barrier impairment is a consistent finding in new MS lesions detected with MRI. Gd-DTPA is a useful marker of new and biologically active lesions and should prove of value in monitoring therapeutic trials in MS.

INTRODUCTION

Magnetic resonance imaging (MRI) is a highly sensitive technique for demonstrating brain lesions in patients with multiple sclerosis (MS). Abnormalities are reported in 85 to 100% of patients with clinically definite MS (Young *et al.*, 1981; Lukes *et al.*, 1983; Runge *et al.*, 1984; Sheldon *et al.*, 1985; Ormerod *et al.*, 1987), and the MRI lesions have been correlated with plaques at postmortem (Stewart *et al.*, 1984; Ormerod *et al.*, 1987). The characteristic pattern is one of multifocal white matter lesions, many of which are adjacent to the lateral ventricles.

At present it is not possible to determine the age or state of biological activity of a plaque seen at a single MRI examination. Acute or chronic lesions (as judged from serial studies) are seen as similar regions of high signal on conventional spin echo (SE) images. One study of brainstem lesions found that the T_1 and T_2

Correspondence to Professor W. I. McDonald, Institute of Neurology, Queen Square, London WC1N 3BG, England.

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relaxation times were generally higher in acute lesions (Ormerod *et al.*, 1986), but the range of values was wide and it was not possible to determine the age from an individual reading. It would be of value to establish lesion age in 2 groups. (1) Patients with a single clinical episode typical of demyelinating disease (e.g., acute optic neuritis) and MRI evidence of asymptomatic multifocal brain lesions. Evidence of lesions of different ages in such patients would suggest a multiphasic disease (i.e., MS); if all lesions were the same age, a monophasic disease (i.e., acute disseminated encephalomyelitis) would be more likely. (2) MS patients participating in therapeutic trials, where the appearance of new and active lesions would provide evidence of continuing disease progression.

Several lines of evidence suggest that the blood-brain barrier (BBB) is abnormal in the early stages of the MS lesion. First, pathological studies of the acute plaque often show a close relationship to cerebral venules, a prominent perivascular infiltrate of lymphocytes (Adams, 1977; Allen, 1984), and fibrinous exudation (Adams *et al.*, 1985); conversely, the chronic plaque is relatively acellular and is dominated by astrocytic gliosis (Lumsden, 1970; Adams, 1977). Secondly, in vivo observations of BBB abnormalities in MS with iodine-enhanced CT scanning have shown that (1) enhancement is often present in an area which is appropriate to recent clinical relapse (Sears *et al.*, 1981); (2) enhancing lesions (symptomatic and asymptomatic) are seen most often at times of clinical relapse (Lebow *et al.*, 1978; Sears *et al.*, 1978; Harding *et al.*, 1978; Reisner and Maida, 1980; Viñuela *et al.*, 1982; Drayer and Barrett, 1984) and (3) enhancement is transient, usually lasting several weeks to months (Aita *et al.*, 1978; Morariu *et al.*, 1980) and may be reversed within hours by corticosteroids (Troiano *et al.*, 1984).

While serial CT studies show that BBB abnormalities correlate well with clinical disease activity, they do not establish the *age* of the enhancing lesions. Because unenhanced CT has a low sensitivity for detecting plaques, it is not possible to state whether a new enhancing lesion represents reactivation of an old plaque or the development of a new one.

MRI, with its high sensitivity in detecting plaques, is well suited to a serial study of BBB abnormalities in MS. As with CT, contrast agents are required to demonstrate BBB impairment with MRI. The most useful to date is gadolinium-DTPA (Gd-DTPA), a paramagnetic agent which strongly enhances the relaxation of water protons (Gadian *et al.*, 1985). In tissues, Gd-DTPA has a greater effect on T_1 than T_2 relaxation. This is because T_2 relaxation is dominated by interactions between water and macromolecules; in the concentrations found in tissues, Gd-DTPA has little additional effect. T_1 relaxation, on the other hand, is less dominated by the presence of macromolecules. T_1 relaxation processes are relatively inefficient and the presence of small amounts of Gd-DTPA can have a considerable effect. Consequently, T_1-weighted sequences (e.g., $IR_{2000/40/500}$ or $SE_{500/40}$) are best to show contrast enhancement. T_1 shortening will produce regions of high signal.

Gd-DTPA enhancement is a normal finding in the pituitary gland and infundibulum, cavernous sinus, choroid plexus and some dural vessels (Brant-Zawadzki *et al.*, 1986; Kilgore *et al.*, 1986). Enhancement within the normal brain parenchyma is not seen, but is reported in a wide variety of intrinsic and extrinsic pathologies (Carr *et al.*, 1984; Berry *et al.*, 1986; Brant-Zawadzki *et al.*, 1986). Gd-DTPA enhancement has proved useful in demonstrating meningiomas (Bydder *et al.*, 1985), small acoustic neuromas (Curati *et al.*, 1986) and other extrinsic tumours (Berry *et al.*, 1986).

Gd-DTPA enhancement of MS plaques has also been seen (Grossman *et al.*, 1986; Guilleux *et al.*, 1986). Grossman *et al.* found that, as with CT, there was a good correlation of the site of enhancing lesions with recent clinical relapses, and more enhancing lesions were seen in patients with clinically active disease; Gd-DTPA-enhanced MRI was also more sensitive than high dose iodine-enhanced CT in detecting BBB abnormalities.

Our main aims in performing a serial study with Gd-DTPA enhanced MRI in MS were to establish (1) the duration of enhancement of MS plaques and (2) the relationship of enhancement to the age of the lesion, in particular whether new lesions appearing on an unenhanced follow-up scan show enhancement.

In view of a previous report that acute lesions have longer relaxation times than chronic ones (Ormerod *et al.*, 1986), we also recorded these times in enhancing and nonenhancing lesions. The T_1/T_2 ratio was also measured as experimental work has shown that it may differentiate pathological features such as oedema and gliosis which are commonly seen in MS plaques (Barnes *et al.*, 1987, 1988).

METHODS

Ten patients aged 28 to 47 yrs with clinically definite MS (Poser *et al.*, 1983) were studied; 8 were in an acute clinical relapse at the time of the first scan, 1 had a relapse 3 months before the first scan; another had had gradual deterioration over several months. All were enrolled in the study after giving their informed consent. MRI was performed on a Picker 0.5T superconducting unit provided by the Multiple Sclerosis Society of Great Britain and Northern Ireland. Follow-up scans were performed approximately 1 month and 6 months after the first scan. Eight patients completed all 3 scans; 1 declined a second and third scan owing to claustrophobia experienced on the first occasion; another declined a third scan as there had been relapses within a few weeks following his previous scans.

Contiguous 10 mm axial slices of the brain were obtained using 4 sequences: $SE_{2000/40}$, $SE_{2000/120}$, $SE_{500/40}$, $IR_{2000/40/500}$. The sequences were performed on each occasion before and after the administration of Gd-DTPA 0.2 mmol/kg (Schering AG) This provided 2 T_1-weighted sequences well suited to demonstrate contrast enhancement ($SE_{500/40}$, $IR_{2000/40/500}$).

From 3 sequences ($SE_{2000/40}$, $SE_{2000/120}$, $IR_{2000/40/500}$), computed images of T_1 and T_2 were obtained using algorithms provided by the manufacturer. From these images, relaxation times were measured in lesions, and in the normal appearing white matter and cortical grey matter in the frontal, temporal, parietal and occipital lobes. Readings were taken from the same regions in the pre- and postcontrast images.

The cervical cord was imaged in 1 patient with a spinal relapse, using a surface receiver coil provided by the manufacturer and an $SE_{500/40}$ sequence.

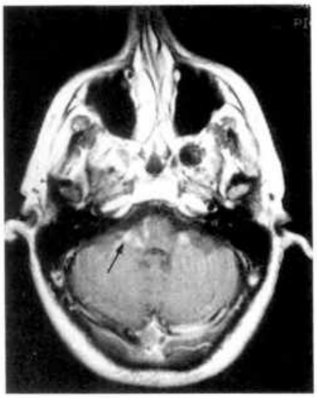

FIG. 1. T_1-weighted image ($SE_{500/40}$) postcontrast. The patient was in an acute relapse with right-sided deafness and ataxia. There is an extensive enhancing right pontine lesion with involvement of the auditory nerve root entry zone (arrowed).

RESULTS

First scan

In all 10 patients unenhanced MRI demonstrated multifocal mainly white matter brain lesions typical of MS. In total, 56 of the lesions showed contrast enhancement. Enhancing lesions were seen in 8 patients: the 2 with no enhancing lesions were not in an acute relapse at the time of scanning. Enhancing lesions in a site compatible with the acute clinical relapse were seen in 6/8 patients (figs 1, 2A). Most enhancing lesions were asymptomatic. Enhancement most often involved the whole lesion, but occasionally there was peripheral ring enhancement only (fig. 3A). In one lesion there was a serpiginous nonenhancing halo beyond the enhancing rim (fig. 3B).

Lesion enhancement was best seen on the T_1-weighted sequences, and of these the $SE_{500/40}$ sequence was more sensitive than $IR_{2000/40/500}$. Some enhancement

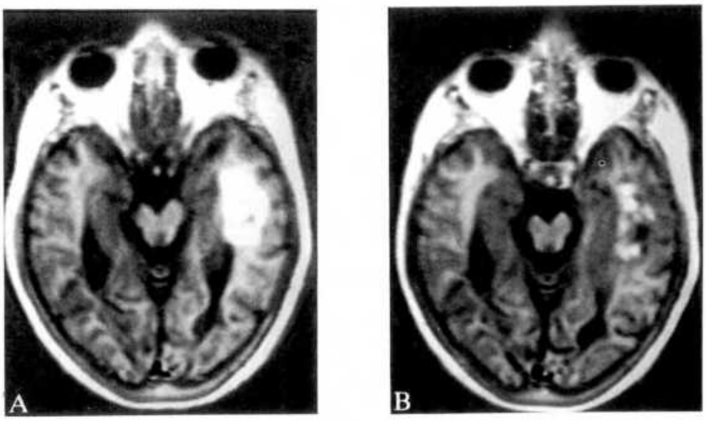

FIG. 2. Postcontrast inversion recovery images ($IR_{2000/40/500}$). A, first scan during an acute relapse with prominent memory disturbance showing a large enhancing lesion in the left temporal lobe; B, second scan after 3 weeks showing persisting but diminished enhancement.

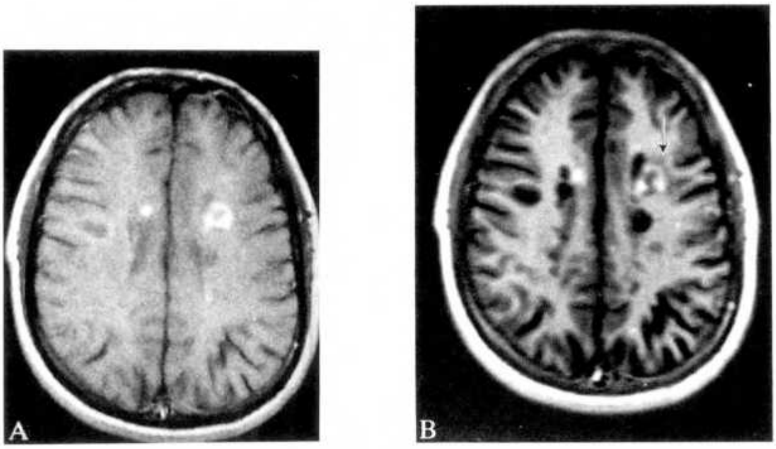

FIG. 3. Postcontrast T_1-weighted images (A) $SE_{500/40}$ and (B) $IR_{2000/40/500}$. There is a ring enhancing lesion, best seen in A, adjacent to the left frontal horn. The inversion recovery image in B shows a nonenhancing region beyond the enhancing ring (arrowed).

was seen on the $SE_{2000/40}$ sequence and sometimes even on the T_2-weighted sequence ($SE_{2000/120}$).

Fig. 4 shows the distribution of relaxation time measurements of enhancing (n = 33) and nonenhancing (n = 71) lesions. The range of values T_1, T_2 and T_1/T_2 seen in both groups was wide and overlapping. It is notable, however, that some nonenhancing lesions had very long relaxation times, particularly T_2, with low T_1/T_2 ratios (less than 2.5).

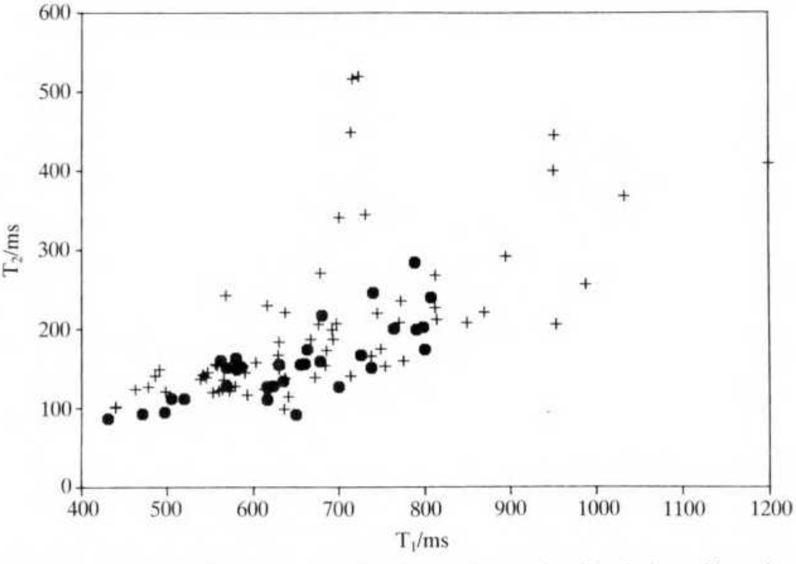

FIG. 4. Distribution of T_1 and T_2 values of enhancing and nonenhancing lesions. Nonenhancing (cross); enhancing (closed circle).

TABLE 1 EFFECT OF Gd-DTPA ON RELAXATION TIMES (in ms) OF MULTIPLE SCLEROSIS LESIONS

			Precontrast	*Postcontrast*	
Enhancing	T_1	Mean	646	392	$P < 0.0001$
		(Range)	(431–807)	(257–507)	
	T_2	Mean	154	152	n.s.
		(Range)	(87–284)	(96–264)	
Nonenhancing	T_1	Mean	693	681	n.s.
		(Range)	(440–1200)	(440–1490)	
	T_2	Mean	198	188	n.s.
		(Range)	(99–519)	(91–490)	

n.s. = not significant.

Following the administration of Gd-DTPA the T_1 of enhancing lesions was shortened by a mean of 39.4% ($P < 0.0001$, ANOVA, Table 1). There was no significant change in T_2 of enhancing lesions and neither T_1 nor T_2 were significantly changed in nonenhancing lesions. Gd-DTPA did not alter the T_1 or T_2 of normal appearing white matter, nor the T_2 of cortical grey matter, but the T_1 of cortical grey matter was significantly shortened ($P < 0.01$, ANOVA, Table 2).

Second scan

This was performed in 9 patients 3 to 5 weeks after the first scan. Of the total of 54 enhancing lesions seen in these patients on the first scan, 42 were no longer enhancing while 12 showed persisting but sometimes diminished enhancement (figs 2, 5).

Twelve new lesion areas (8 new lesions and 4 definite areas of extension of old

TABLE 2. EFFECT OF Gd-DTPA ON RELAXATION TIMES (in ms) OF NORMAL-APPEARING BRAIN

			Precontrast	*Postcontrast*	
White matter	T_1	Mean	388	387	n.s.
		(SD)	(39)	(35)	
	T_2	Mean	83	81	n.s.
		(SD)	(5)	(6)	
Grey matter	T_1	Mean	565	549	$P < 0.01$
		(SD)	(35)	(32)	
	T_2	Mean	100	99	n.s.
		(SD)	(7)	(8)	

n.s = not significant.

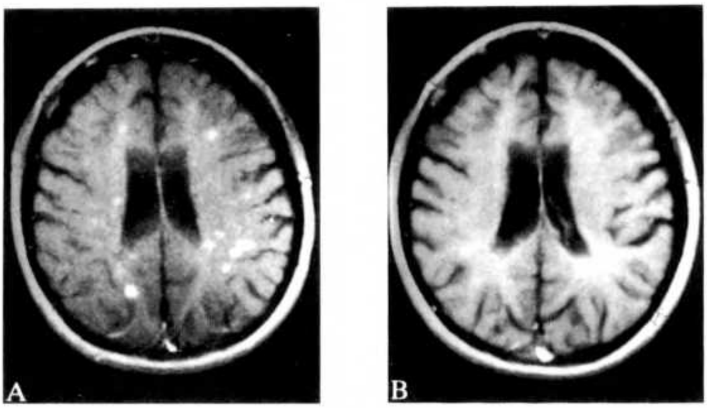

FIG. 5. Postcontrast T_1-weighted images ($SE_{500/40}$). The initial scan (A) shows many enhancing lesions, none of which are still enhancing at the follow-up scan (B) 4 weeks later. (Slice B is at a slightly different level from slice A, but the adjacent follow-up slice also showed no enhancement.)

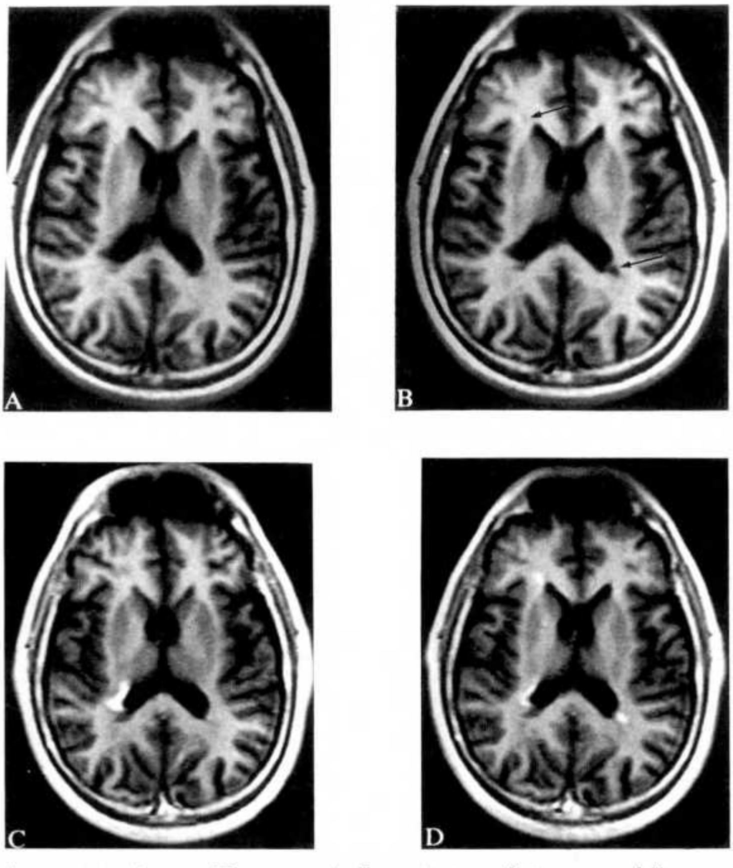

FIG. 6. Inversion recovery images ($IR_{2000/40/500}$). Precontrast: A, first scan, B, follow-up (4 weeks later). Postcontrast: C, first scan, B, follow-up. Two new lesions appear on the unenhanced follow-up scan (B, arrowed), and both display contrast enhancement (D).

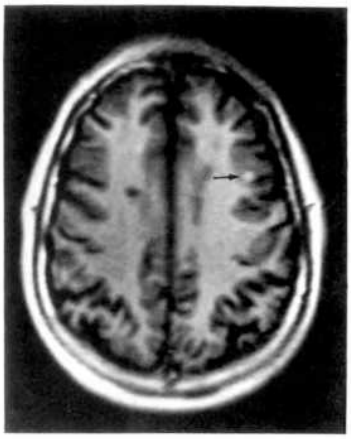

FIG. 7. Postcontrast inversion recovery images ($IR_{2000/40/500}$). There is a small enhancing lesion in the left frontal cortex (arrowed). This was only visible postcontrast.

lesions) were identified on the unenhanced scan, and all displayed enhancement (fig. 6). Enhancement was also seen in 2 older lesions which on the first scan were nonenhancing.

Third scan

Eight patients were rescanned 6 months after the first scan. A total of 15 new lesion areas were seen in the unenhanced scan, of which 8 displayed enhancement. Enhancement was also seen in 2 older lesions which had been nonenhancing on earlier scans. No enhancement was seen in any of the lesions which had enhanced on earlier scans.

Lesions seen only with Gd-DTPA enhancement

Over the course of the study a total of 10 small enhancing areas were seen which were not visible on the unenhanced study. In each case they were seen in the cortex or subcortical white matter, and were no longer visible on follow-up scan (fig. 7).

Spinal cord

One patient with an acute spinal relapse showed an extensive area of enhancement in the cervical cord (fig. 8).

Tolerance to Gd-DTPA

No side effects were noted in association with Gd-DTPA administration. The relapses which occurred in 1 patient within several weeks of the scan were probably coincidental.

A transient elevation in the serum iron level was seen in 8/27 examinations 4 to 24 h after injection, and returned to normal in all cases within 48 h.

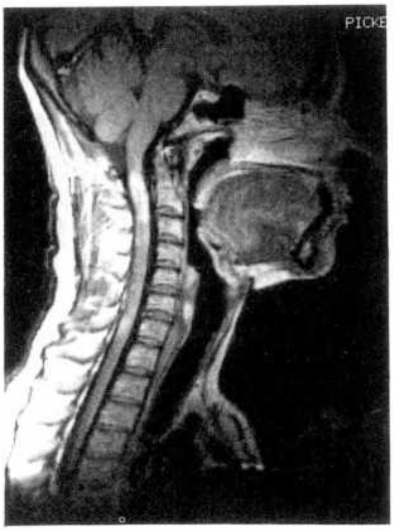

FIG. 8. Postcontrast T_1-weighted image ($SE_{500/40}$) of the cervical cord in a patient with an acute cervical myelopathy. There is extensive patchy enhancement of the cord, especially at C1 and C2.

DISCUSSION

The study confirms that Gd-DTPA enhanced MRI frequently demonstrates enhancing lesions in patients with clinically active MS. The majority of enhancing lesions were asymptomatic. Most lesions enhanced for less than 1 month, and none for as long as 6 months. Enhancement was seen in every new lesion which appeared in a period of approximately 1 month (between the first and second scans), and in many but not all lesions that appeared in a period of 5 months (between the second and third scans).

Several lines of evidence suggest that vascular events in MS can occur independently of demyelination. First, perivascular infiltrates in the retina, a region free of myelin and oligodendrocytes, have been found in 24% of patients with acute optic neuritis (Lightman *et al.*, 1987) and are associated with a significantly greater risk of developing MS. Secondly, perivascular cuffs (Adams, 1977) and inflammatory infiltrates confined to vein walls (Adams *et al.*, 1985) have been reported distant from plaques in the normal appearing white matter in MS. Occasionally, perivascular cuffs can be traced from a region of active myelin breakdown at the edge of a plaque into adjacent normal appearing white matter (McDonald, 1983). These observations raise the question as to whether the vascular changes are the primary—or at least a necessary early—event in the development of new lesions. The present study supports this hypothesis in demonstrating that BBB impairment is a consistent feature of recognizably new lesions or new parts of existing lesions.

The study also showed that the BBB impairment develops occasionally in older previously nonenhancing plaques without evidence of an increase in size. This perhaps is not surprising: if vascular mechanisms are of primary importance, reactivation of BBB abnormalities in older plaques may be expected to occur occasionally. It is of interest, however, that this was seen in only 4 out of the very large number of nonenhancing plaques followed serially. Gd-DTPA enhancement appears most often to occur in new lesions.

Pathological studies indicate that demyelination begins in a perivenular distribution, and spreads centrifugally (Lumsden, 1970). Active demyelination is often seen at the edge of the plaque (Prineas and Connell, 1978), and this may well correspond to the ring enhancing lesions which were noticed in the present study and by Grossman *et al.* (1986). A second concentric nonenhancing halo seen in 1 lesion beyond the enhancing ring may reflect oedema surrounding a particularly large and active plaque.

Cortical plaques are seen frequently at necropsy (Brownell and Hughes, 1962; Lumsden, 1970), but relatively infrequently with unenhanced MRI. Lesions in this region are less easily seen because of reduced contrast due to the higher T_1 and T_2 of the adjacent grey matter and CSF. It is not surprising then that enhanced MRI demonstrated a number of small cortical or subcortical lesions that were otherwise invisible.

Cortical grey matter perfusion is 4 times that of white matter so that the intravascular concentration of Gd-DTPA will be considerably higher in the cortex. This concentration is probably sufficient to account for the small but statistically significant fall in the T_1 of normal appearing cortical grey matter following administration of Gd-DTPA.

As the ranges of T_1, T_2 and T_1/T_2 for enhancing and nonenhancing lesions were wide and largely overlapping, we conclude that relaxation times are generally not useful in determining the age or activity of lesions. They may, however, help to clarify the pathology of the lesions.

Lesions are seen on NMR images by virtue of an increase in water proton content, which produces an elevation of T_1 and T_2. The degree of elevation in T_1 is positively correlated with the total amount of water in a lesion. The degree of T_2 elevation also depends on the total amount of water as well as on the macromolecular environment of the water. The presence of macromolecules such as protein will shorten T_2 but not T_1. In other words, a high water content increases both T_1 and T_2, but in the presence of macromolecules the relative increase in T_2 is less. Barnes *et al.* (1987) have confirmed this effect experimentally in demonstrating a higher T_1/T_2 ratio in vasogenic protein-rich oedema when compared with protein-free oedema.

Enhancing plaques have an impaired BBB, and thus may be expected to contain vasogenic protein-rich oedema. Adams *et al.* (1985) have indeed shown that a fibrin-rich exudate is often present in acute plaques. Such protein-rich oedema probably accounts for the elevated T_1 and T_2 and relatively high T_1/T_2 ratio of enhancing lesions, although an increase in intracellular water due to cellular infiltration may also contribute.

Astrocytic gliosis is the striking pathological feature of the chronic plaque and becomes established within a few months of the appearance of a lesion (Adams, 1977; Allen, 1984). It is likely then that gliosis is present in many nonenhancing lesions. Experimentally induced gliosis following a cold lesion in the cat produces modest elevations in T_1 with little elevation in T_2, and hence a high T_1/T_2 ratio (Barnes *et al.*, 1988). Gliosis per se then, is not an adequate explanation for the clearly elevated T_2s and low T_1/T_2 ratios seen in some nonenhancing lesions. Such values indicate a high water content which is probably extracellular and of low macromolecular content (Barnes *et al.*, 1987), and indeed marked protein free expansion of the extracellular space has been reported in chronic plaques (Périer and Grégoire, 1965).

What is the origin of such oedema in nonenhancing lesions? One possibility is transependymal movement of CSF into the brain which would produce a high T_2 in periventricular plaques. However, such a process has only been demonstrated when the intraventricular pressure is raised (i.e., in hydrocephalus) and would in any case be unlikely to produce the elevated T_2 seen in lesions distant from the ventricles. Alternatively, there might be an abnormality of the BBB which is not detected with Gd-DTPA. There is some evidence that the BBB remains abnormal in more chronic plaques. First, supravital injections of trypan blue into the cerebral circulation have shown BBB impairment in chronic as well as acute plaques, though the impairment was less marked in chronic lesions (Broman, 1949, 1964). Secondly, vessel wall abnormalities are seen in both acute and chronic plaques, consisting predominantly of inflammation in the acute phase and repair in the chronic phase (Adams *et al.*, 1985). It is possible that with incomplete repair to the vessel wall after the acute phase, a selective leak of water and small molecules continues, while larger molecules such as protein and Gd-DTPA can be retained.

The main conclusion from the present study is that Gd-DTPA is a sensitive marker of the BBB impairment which occurs consistently in new plaques. As such it should prove useful in monitoring therapeutic trials in MS.

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PROCEDURAL LEARNING AND NEOSTRIATAL DYSFUNCTION IN MAN

by J. A. SAINT-CYR[1,2,3,5], ANN E. TAYLOR[1,5] *and* A. E. LANG[1,4,6]

(From the [1]Playfair Neuroscience Unit, Departments of [2]Anatomy, [3]Psychology and [4]Medicine, University of Toronto; [5]Department of Psychology and [6]Department of Medicine, Division of Neurology, Toronto Western Hospital, Canada)

SUMMARY

Patients with early stage Parkinson's disease are shown to be selectively impaired in a cognitive task of procedural learning while remaining intact in recall and recognition tests of declarative memory. In contrast, amnestic patients showed the opposite set of deficits, thus demonstrating a double dissociation. Patients with early Huntington's disease were either comparable to the parkinsonian patients or to amnestics. In the advanced Huntington's group, both procedural learning and declarative memory were impaired. It is argued that cognitive procedural learning depends on the establishment of heuristic strategies through the action of a circuit which involves the neostriatum and the prefrontal cortex.

INTRODUCTION

In recent years a distinction has been made between two functionally and supposedly anatomically separable memory systems in man. Declarative memory, the most intensively studied, is the ability to store and consciously recall or recognize data in the form of words, visual images, or events (Tulving, 1983; Squire, 1986). In contrast, procedural learning is the ability gradually to acquire a motor skill or even a cognitive routine through repeated exposure to a specific activity constrained by invariant rules (Cohen and Squire, 1980). In the latter, strategies, applicable to changing stimulus configurations within a task, must be acquired through practice. Individual moves are not memorized nor can the successful sequence be consciously recalled. Acquisition of such motor skills or cognitive routines is implicit, manifest in significantly reduced reaction times or error scores over trials. While the product of the declarative system is a *memory*, some authors consider the product of the procedural system a *habit* (Mishkin *et al.*, 1984).

Correspondence to Dr J. A. Saint-Cyr, Department of Psychology, Edith Cavell Wing, Suite 4-022, Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, Canada M5T 2S8.

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The dissociation of these two systems has been suggested by the demonstration of spared procedural learning in patients rendered amnestic for declarative information following selective lesions of temporal lobe and/or anatomically-related midline thalamic structures (Zola-Morgan *et al.*, 1982; Squire *et al.*, 1984). For example, cases H.M. and S.S., and patients with alcoholic Korsakoff's syndrome are able to acquire new skills such as mirror reading or drawing and pursuit rotor tracking despite their inability to recall the experience (Milner, 1962; Corkin, 1965; Weiskrantz and Warrington, 1979; Baddeley *et al.*, 1982; Corkin *et al.*, 1985; Warrington, 1986). Under certain testing conditions, even the most profoundly amnestic patient, H.M., is reported to have acquired the procedures necessary to solve the cognitively demanding Tower of Hanoi puzzle (Cohen *et al.*, 1985). In all such amnestic cases the underlying pathology involves limbo-diencephalo-cortical circuits associated with the hippocampus and amygdala (Scoville and Milner, 1957) or the mamillary bodies and midline thalamus as in Korsakoff's syndrome (Victor *et al.*, 1971). In case N.A., who has a selective verbal memory deficit but has demonstrated proficiency on the Tower of Hanoi (Squire and Moore, 1979), MRI revealed involvement not only of the originally documented left medial dorsal nucleus but also of the mamillary bodies and right temporal pole (Squire *et al.*, 1987). Experiments in subhuman primates have confirmed the importance of the subcortical structures mentioned above in recognition memory, one of the important subroutines within the declarative system (Mishkin, 1982).

Although the anatomical substrate of the 'spared' procedural system in amnesia has not been established, the neostriatum (especially the caudate nucleus) is proposed to play a key role (Mishkin *et al.*, 1984; Butters *et al.*, 1985). Martone *et al.* (1984) initially explored this hypothesis by comparing patients with Huntington's disease (HD), in which there is structural damage to the neostriatum, with amnestic Korsakoff's patients with damage to the medial dorsal thalamic nuclei and the mamillary bodies. Both groups were administered a test of mirror reading competence and a later recognition test to probe memory for frequently recurring words. Even advanced HD patients, although significantly less efficient in acquiring the mirror reading procedural skill, remained near-normal on the declarative task of word recognition. Korsakoff patients exhibited the opposite profile, demonstrating a double dissociation between the two memory systems in these two groups.

On further testing, both advanced HD and amnestic patients with limbodiencephalic pathology (i.e., 5/6 Korsakoff patients and case S.S.) were unable to solve the cognitively more complex Tower of Hanoi puzzle, previously heralded as an example of procedural learning (Butters *et al.*, 1985). In contrast, early HD patients could master this task. The failure to obtain the expected dissociation was later attributed to task contamination by problem-solving elements. It was concluded that the tower format is not appropriate clearly to demonstrate differential ability in cognitive procedural learning in these pathologically distinct groups.

While task contamination in the form of a problem-solving load may have contributed to the lack of experimental effect, an issue crucial to the interpretation of these findings may be the full extent of pathology in HD and Korsakoff's syndrome. In both instances, this eventually involves the cortex (Forno and Norville, 1979; Butters and Cermak, 1980; Kuhl *et al.*, 1982). Indeed at a cortical level, damage or dysfunction may well implicate common areas which are important to cognitive as opposed to motor procedural learning, such as the prefrontal region, either directly through cell loss or, at the least, indirectly through dysfunctional input from affected midline thalamic structures, namely the nucleus medialis dorsalis. Recent cerebral blood flow studies in early HD patients have revealed reduced flow in frontotemporal regions (Tanahashi *et al.*, 1985), while reduced oxygen and glucose metabolism have been observed in the frontal lobe (Leenders *et al.*, 1986). On this basis, no dissociation between basal ganglia patients and Korsakoff amnestic patients on cognitively-loaded tasks of procedural learning would be expected.

These considerations prompted reinvestigation of the original hypothesis implicating the neostriatum in procedural learning. However, selection of patient groups with restricted pathology was mandatory. Moreover, it appeared essential to develop an experimental strategy which would depend exclusively on practice for mastery without over-reaching the problem-solving resources of the patient groups selected. Accordingly, individuals in the early stages of Parkinson's disease (PD) were recruited as the best available model of regional basal ganglia dysfunction (Marsden, 1982), while amnestic patients were limited to those with no behavioural or radiological evidence of disturbance to prerolandic cortical areas. Early and advanced HD patients were also included to permit comparisons with previous studies.

The present investigation describes a selective deficit of procedural learning in parkinsonian patients and in a subgroup of early HD patients and suggests that the basal ganglia play a critical role in this type of cognitive operation. A double dissociation is demonstrated with amnestic patients who are selectively impaired in declarative memory tasks. Other patients with HD had varying degrees of impairment in both procedural and declarative functions.

SUBJECTS AND METHODS

Twenty-four patients in the early stages (I and II, Hoehn and Yahr, 1967) of PD were studied. Of these, 13 were receiving levodopa with a peripheral decarboxylase inhibitor (Sinemet), 3 were taking bromocriptine and 3 amantadine in combination with either Sinemet or bromocriptine. One patient was taking amantadine alone while a single patient was on anticholinergic medication. The remaining 3 patients were unmedicated. Demographic data are presented in Table 1. All these patients performed comparably in all tests, failing to suggest any medication effect.

Twelve patients with untreated (HD), 8 early (HDE) and 4 in a more advanced stage of disease (HDA), were also included. The HD patients were separated on the basis of clinical severity as measured by the Shoulson and Fahn scale (1979) and the magnitude of decline in intellectual function. HDA was defined as a score of 10 or below on the Shoulson and Fahn scale and a

TABLE 1 DEMOGRAPHICS

Group	*n*	*Age (yrs)*	*NART-VIQ*	*WAIS-R VIQ*	*Purdue*	*Duration (yrs)*	*Stage*	*Trt*	*Lat*
PD	24	60 (12)	114 (8)	111 (11)	37 (8)	4 (2)	I = 17 II = 5 III = 2	0 = 3 1 = 21	R = 12 L = 12 B = 3
NC	24	61 (12)	115 (8)	112 (11)	49 (6)	—	—	—	—
HDE 1	4	31 (12)	108 (9)	92 (13)	44 ( )	1 (0)	13 (0)	0 = 4	—
HDE 1-NC	4	33 (14)	113 (5)	115 (5)	—	—	—	—	—
HDE 2	4	40 (6)	108 (6)	94 (8)	40 ( )	2 (1)	13 (1)	0 = 4	—
HDE 2-NC	4	39 (10)	113 (5)	111 (7)	—	—	—	—	—
HDA	4	53 (7)	112 (10)	82 (13)	26 (6)	4 (3)	19 (3)	0 = 4	—
HDA-NC	4	50 (7)	110 (7)	52 (4)	52 (4)	—	—	—	—
					WMS-MQ				
AM	2	27 (6)	—	118 (13)*	68 (12)	—	—	—	—
AM-NC	2	26 (3)	—	115 (13)	115 (15)	—	—	—	—

NC = normal controls; PD = Parkinson's disease; HDE 1 = early Huntington's disease, group 1, HDE 2 = early HD, group 2; HDA = advanced HD; AM = amnestic patients. *Raven's Standard Progressive Matrices—determined IQ score. WMS-MQ = Wechsler Memory Scale, Memory Quotient. NART-VIQ = estimated premorbid verbal IQ from New Adult Reading Test (Nelson and O'Connell, 1978). Purdue = number of pegs placed with each hand and both together in the Purdue Pegboard Test. Stage = according to Hoehn and Yahr (1967) for PD and Shoulson and Fahn (1979) score for HD (13 = normal, highest score possible). Trt = treatment for groups: 0 = no treatment; 1 = good response to conventional pharmacotherapy. Lat = laterality of symptoms at onset: R = right; L = left; B = bilateral. NB. All data are means with SD in parentheses.

Wechsler (Wechsler, 1981) verbal IQ score at least 20 points below the estimated premorbid level as measured by a reading test (Nelson and O'Connell, 1978) (*see* Tables 1, 2).

Patients in both basal ganglia groups were assessed and rated for stage and disability by the same neurologist (A.E.L.) using techniques in common clinical practice (Canter *et al.*, 1961; Hoehn and Yahr, 1967; Duvoisin, 1971; Shoulson and Fahn, 1979; Marsden and Schacter, 1981). In addition, all patients and certain control subjects were assessed with the Purdue Pegboard Test of manual speed and dexterity. Performance on this instrument is well correlated with clinical tests of severity of motor impairment in PD, providing a measure of motor system status at the moment of neuropsychological testing (Taylor *et al.*, 1986).

Two patients with severe declarative memory impairment represented limbo-diencephalo-cortical amnesia. One (H.E.) had suffered herpes encephalitis 5 yrs previously resulting in profound generalized loss for the recall of new information. This patient was comparable to a previously described case of postencephalitic amnesia (Cermak and O'Connor, 1983). The other amnestic patient (L.D.) had an apparently congenital learning disability characterized by lifelong documented memory deficits of both a verbal and visual nature with concomitant dyslexia and spelling impairment. CT scan results in patient H.E. reflected involvement of both temporal lobes during the acute phase of the illness (i.e., while in hospital with active encephalitis). In patient L.D , the CT scan revealed focal atrophy or dysgenesis of left temporal and right parietal areas. Severity of amnesia in these 2 patients was measured by the Wechsler Memory Scale (Wechsler and Stone, 1945). Memory quotients (WMS-MQ) of 59 (H.E.) and 77 (L.D.) when compared with IQ scores of 106 and 130 respectively, revealed severe impairment in the declarative system (*see* Table 1).

All subjects, apart from the amnestics, received the verbal section of the Wechsler Adult Intelligence Scale (Revised) (WAIS-R) (Wechsler, 1981) and the New Adult Reading Test (NART, Nelson and O'Connell, 1978) as part of a standardized screening battery (Taylor *et al.*, 1985). The performance section of the WAIS-R was not included due to potential contamination from motor and time demands embedded in most subtests. PD and HD patients were simultaneously matched to control subjects on the basis of age and NART score. Amnestic patients were rated and matched in intelligence with control subjects with the Raven's Progressive Matrices (Raven *et al.*, 1978) as mild dysphasia in H.E. and lifelong academic problems in L.D. precluded valid estimates with the verbal section of the WAIS-R.

All participants gave informed consent and none had evidence of intercurrent disease. Demographic data with respect to matching of patients and controls, disease duration, stage, symptom severity as measured by the Purdue Pegboard or WMS-MQ, and lateralization of initial symptoms for the PD group are presented in Table 1. The symptom profile for the HD patients is presented in Table 2.

Tests of declarative memory

These included the following. (1) The Logical Passages (LP) of the Wechsler Memory Scale scored in full and half credits (Powers *et al.*, 1979). (2) The Buschke Selective Reminding Test (BSRT), a standard declarative test of verbal supraspan learning (reduced to 6 repetitions of 12 words for this older population) (Buschke, 1973). The score was the total number of words recalled. (3) Recognition span for words, images and spatial position was assessed by a delayed non-match-to-sample paradigm (DRT) (Taylor *et al.*, 1986) adapted from Albert and Moss (1984). In the current version, the stimuli are presented in pamphlet form and a letter cancellation task given during the intertrial interval as a distractor.

Tests of procedural memory

In consideration of the elderly PD population, a simplified variant of the Tower of Hanoi puzzle (Cohen *et al.*, 1985) was developed and administered to all subjects. The newly-devised Tower of Toronto served as the measure of cognitive procedural learning and is fully described in the Appendix. In brief, a colour rather than size coding principle is used, and 3 discs (in TO) or 4 discs

TABLE 2 CLINICAL PROFILES FOR HD PATIENT GROUPS

Group	*Case*	*Age at onset**	*Duration (yrs)*	*1st symptom*	*Chorea score*	*Purdue*	*S and F*	*Psychiatric*	*Dem*
HDE 1	1	21	0.75	Chorea	8	42	13	Irritable	14
	2	22	1.5	Chorea	12	47	13	Poor insight	9
	3	27	1	Chorea	10	40	13	Irritable	8
	4	48	1	Chorea	7	46	13	Depression	11
HDE 2	1	29	4	Chorea	16	20	12	Poor concentration	2
	2	34	0.5	Mood change	0	56	12	Poor concentration/ mood	13
	3	35	1	Chorea	10	42	13	Cognitively dull Poor sleep	16
	4	54	1	Chorea	16	41	13	Poor insight	16
HDA	1	39	3	Chorea	12	18	7	Irritable/poor concentration	31
	2	50	5	Ataxia	16	26	6	Inappropriate affect	23
	3	51	6	Chorea	19	27	10	Depression/ poor insight	25
	4	57	1	Chorea	22	33	7	Poor insight/ anxiety	27

* May be underestimated due to subtlety of early symptoms, denial and poor insight. Chorea score from Marsden and Schacter (1981). Purdue scores are total number of pegs placed with each hand separately and together S and F = rating from the Shoulson and Fahn (1979) scale. Psychiatric = complaints and/or personality changes since diagnosis. Dem = index of dementia is fall in IQ comparing NART and current WAIS-R VIQ scores

(in TT) are used rather than 5, in contrast to the Tower of Hanoi. This task was designed to be intermediate in difficulty between the Tower of Hanoi as used by Cohen and his colleagues (Cohen *et al.*, 1985) and the Tower of London devised by Shallice (1982). The need to develop the Tower of Toronto grew out of pilot experiments using the other formats with a wide variety of patients and normal control subjects of all ages. In other testing, amnestic patients were also administered the Wisconsin Card Sorting Test (Heaton, 1981), a classical task of problem solving considered to depend upon the integrity of the prefrontal area (Milner, 1963). This was included to demonstrate retained ability to form, maintain, and switch mental sets in a problem-solving situation in these memory-disordered patients.

Statistical analysis

Results of the declarative and procedural memory tests comparing PD and NC groups were analysed either parametrically or nonparametrically as appropriate. Where sample size was extremely small (n = 4 or less) prima facie evidence of significant differences was inferred where patient scores fell more than 2 SDs beyond normal values. Where appropriate, multiple regression analyses were carried out with a General Linear Models program (Systat Inc., Evanston, IL).

RESULTS

The results are summarized in Tables 3 and 4 and in figs 1 and 2. In comparison with their matched controls, PD patients performed normally on all declarative memory tasks but were impaired on the Tower of Toronto (TT) procedural task. Normal performance was observed on LP, BSRT and DRT and on the preliminary (3 disc) phase of the tower task. However, they demonstrated significantly impaired performance on both blocks (TT1 and TT2) of the 4 disc phase of the Tower of Toronto. The difference between the PD and NC groups would have been even more apparent had the maximum number of moves per trial on TT1 and TT2 not

TABLE 3 PERFORMANCE ON TESTS OF DECLARATIVE MEMORY

			DRT		
Group	*WMS-LP*	*BSRT*	*Spatial*	*Words*	*Designs*
PD	20 (4)	43 (9)	11 (2)	14 (0)	14 (0)
NC	20 (5)	48 (9)	12 (2)	14 (0)	14 (0)
HDE 1	12 (6)*	36 (11)*	11 (1)	13 (1)	13 (2)
NC	22 (4)	57 (3)	12 (2)	14 (0)	14 (0)
HDE 2	19 (3)	49 (3)	13 (1)	14 (0)	13 (1)
NC	18 (4)	51 (7)	13 (2)	14 (0)	14 (0)
HDA	9 (1)*	36 (10)*	6 (0)*	11 (4)	12 (3)
NC	22 (4)	51 (8)	14 (0)	14 (0)	14 (0)
AM	9 (5)*	23 (5)*	4 (1)*	6 (1)*	5 (1)*
NC	27 (8)	62 (9)	14 (0)	14 (0)	14 (0)

Data are mean values with SD in parentheses. * $P < 0.01$ for PD and NC comparisons, a difference of > 2 SD between patient and control means in all other groups. WMS-LP = Logical Passages subtest of the Wechsler Memory Scale. BSRT = Buschke Selective Reminding Test. DRT = Delayed Recognition Span Test. HDE = early Huntington's disease, subgroups 1 and 2. HDA = advanced HD. AM = amnestic patients.

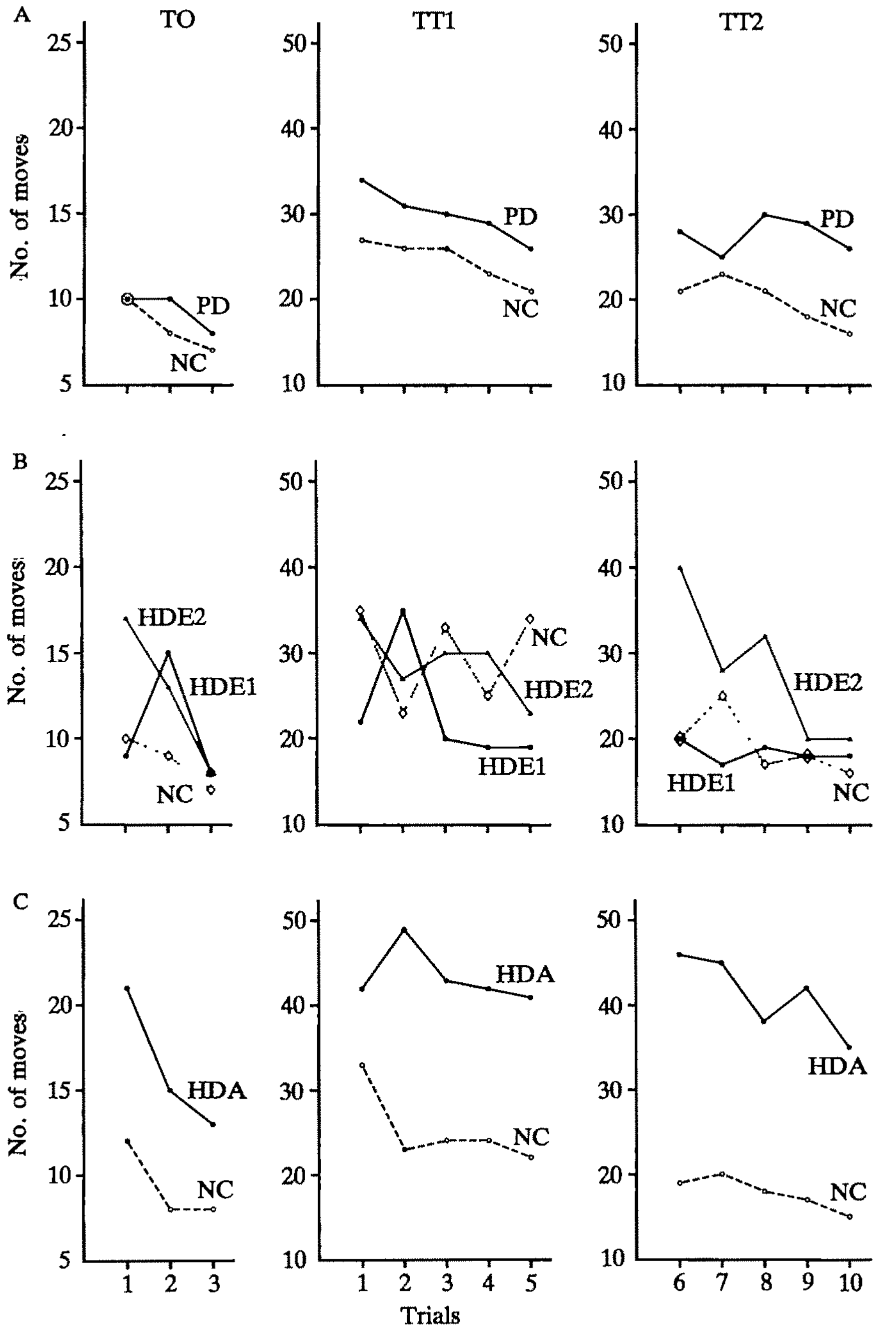

FIG. 1. A, performance of Parkinson's disease (PD), B, both early (HDE 1 and HDE 2) and C, advanced (HDA) Huntington's disease patient groups compared with matched normal control (NC) subjects on the three phases of the Tower of Toronto (TT) task. Scores are plotted for each trial for the 3 disc phase (TO) and for each block (TT1 and TT2) of the 4 disc phase.

In sharp contrast, the amnestic patients were clearly severely impaired in all declarative tests including the simple DRT recognition series for which even HDA patients achieved near-normal scores in 2/3 of the modalities tested (*see* below). However, both amnestic patients performed the 3 disc TO phase optimally and solved the second phase of the Tower of Toronto (TT2) within normal limits. Like their matched controls, amnestic patients stabilized performance at criterion by the end of 10 trials. Transient difficulty with TT1 in the amnestic group can be attributed to either normal variation in this phase (also observed in some NC subgroups) and/or the additional distraction of having constantly to refer to the displayed rules to refresh their memory. NC, PD, and HDE subjects did not require such cueing. In addition, the amnestic patients were well within normal limits on the Wisconsin Card Sorting Test (6 categories).

As a group, the 12 HD patients were somewhat impaired on both procedural and declarative tasks. However, the 8 HDE patients could be divided into two groups based on their performance on the memory battery as a whole. One group of 4 (HDE 1) was more comparable to the amnestic than to PD patients in displaying relative weakness on free recall tests of declarative memory (i.e., Logical passages from the Wechsler Memory Scale, LP; and Buschke Selective Reminding Test, BSRT), while performing normally on the TT1 and TT2 blocks of the Tower of Toronto. Unlike amnestic patients their performance on the Delayed Recognition Test (DRT) was within normal limits. The remaining 4 HDE patients (HDE 2) performed like the PD group in that they were unimpaired on any of the declarative tasks but were worse than their matched controls on TT2. However, they differed significantly from the PD patients in demonstrating marked improvement between trials 6 and 10 of TT2. For the HDA patients, a more profound impairment of declarative memory was accompanied by failure to perform adequately on any phase of the Tower of Toronto test. All tasks, with the exception of the DRT design and word series, were performed extremely poorly by this group.

It is important to note that, before beginning the 4 disc series, the goal of the tower task was clear to all subjects and that the means of achieving it was available to them. This was evident during the 3 disc phase where the optimal solution of 7 steps could be readily visualized after the first or second moves. For PD, HDE 1, NC and amnestic groups, little further experience was necessary once they realized that they could predict the necessary sequence accurately from inspection of the array. However, the addition of a fourth disc, which increased the minimal number of moves to 15, added the dimension of true procedural learning to fundamental problem-solving. During TT1 and TT2 even the most successful subjects had to make approximately 10 moves before foreseeing the move sequence leading to the solution. Successful performance only stabilized for the last 3 trials of TT2, even for NC subjects. No patients or control subjects were able to recall the sequence executed or could articulate their strategy for solution after any individual trial. Only 1 young (24 yrs) NC subject was able eventually to identify the importance of a correct first move by the conclusion of the tenth trial.

The colour-coding feature of the Tower of Toronto may favour different heuristics compared with strategies inherent in the size-coded Tower of Hanoi. Nevertheless, the 4 disc Tower of Toronto, preceded by the 3 disc phase, appears to be a useful variant of the original puzzle. While it certainly involves considerable experience prior to mastery, this can be acquired within one testing session. Moreover, it was not beyond the competence of either the amnestic patients in this study or of the other subject groups which included individuals in the eighth decade (PD and matched NC).

Finally, it was also demonstrated that older subjects required more trials than younger ones (in our current pool of 49 NC subjects, age and TT2 are correlated; $r = 0.434$, $P < 0.002$). This relationship was not found in the PD group. In the total HD group ($n = 12$), a significant correlation with age was also found ($r = 0.641$, $P < 0.025$). Although not found for the NC or PD groups, there was an additional relationship to estimated decline in IQ in the HD patients ($r = 0.701$, $P < 0.01$) and an interaction between age and this fall in IQ ($r = 0.747$, $P < 0.025$). It will be argued in the Discussion that those correlations reflect advancing disease and dementia in HD.

In summary, our experience has been that the Tower of Toronto is a clinically valid and experimentally sound test of cognitive procedural learning.

DISCUSSION

The selective deficit in procedural learning versus declarative memory in the PD patients strongly supports the hypothesis that the neostriatum and associated structures belong to a circuit essential for the normal acquisition of cognitive procedures. This position is strengthened by comparison with the amnestic patients revealing a double dissociation between the declarative memory failures in amnesia and the procedural learning impairment in PD. These findings extend the previous demonstration of a double dissociation between Korsakoff and HD patients which was restricted to cognitively simpler mirror reading and recognition memory abilities (Butters *et al.*, 1985). The present results with PD and amnestic patients are the first report of a double dissociation using a cognitive procedural task.

The situation in early HD is quite complex since two distinct and doubly-dissociated groups could be defined cognitively despite the presumed homogeneity of the underlying pathology as suggested by CT and PET scan studies (Young *et al.*, 1987). In the HDE 1 group, mnestic strengths and weaknesses were quite comparable to the pattern of the amnestic patients, suggesting dysfunction of limbo-diencephalo-cortical circuits. Previous studies have shown decreased cerebral blood flow in frontotemporal regions in HDE patients (Tanahashi *et al.*, 1985). Since a declarative memory impairment may be present even at this stage and certainly accompanies the dementia which evolves with advancing disease, a relationship might be suspected between the decreased temporal cerebral blood flow pattern at onset and the presence of a declarative memory problem.

The disturbance of procedural learning present in early PD suggests that the component of the neostriatum critical to this function normally receives nigral dopaminergic input. The preserved ability in procedural learning, as shown by excellent scores on TT2 in the HDE 1 patients, suggests that caudate pathology, assumed to be present on the basis of symptomatology, may not have reached a critical dysfunctional threshold. Penney and Young (1986) have recently suggested that the striosomes, richly innervated by the dopaminergic fibres arising from the substantia nigra, may not be the first regions of the neostriatum affected in early HD. In some HD patients the striosomes may only become involved at a later stage as shown by reduced D2 receptor binding (*see* Leenders *et al.*, 1986). In those cases, the universally impaired procedural learning of the early PD patients, who are known to have an average 60% loss of caudate dopamine at onset, and the HDE 1 patients, who might have pathological involvement of the matrix but not the striosomal regions, could be reconciled. In contrast, the HDE 2 group would be suspected of earlier rather than later pathological involvement of the dopamine-innervated striosomal regions, thus mimicking early PD with regard to the effects of neostriatal dysfunction on cognitive processes.

Another potentially correlative observation is that the striosomes receive medial prefrontal cortical inputs while the matrix region receives projections from the more lateral frontal and the parietal and temporal association areas (Penney and Young, 1986). This reinforces the hypothesis that procedural learning in man is supported by circuits involving prefrontocaudate striosomal topographic projections. The corollary would be that the declarative memory impairment found in the HDE 1 and HDA groups is related to temporal cortical-caudate (and caudal-ventral putamen; *see* Saint-Cyr *et al.*, 1987) matrix based circuits. The variability in cognitive function in early HD shown in this study can be seen as another example of the well-recognized heterogeneity of presenting symptoms in this disorder which is tacitly assumed to be related to variations in the nature, distribution or the severity of the underlying neuropathological changes.

In advancing HD, deficits would therefore be expected in both the declarative and procedural domains with the progressive pathological involvement of the entire neostriatum and eventual extension into the neocortex. However, the most severely affected region is acknowledged to be the caudate nucleus (Kuhl *et al.*, 1982; Penney and Young, 1986). Indeed, the correlation found with age and TT2 performance in the HD patients as a group may not be attributable to normal ageing alone and an interaction with advancing disease is suggested. Our observations of decline in IQ, worsening of severity according to Shoulson and Fahn ratings and chorea score, associated with catastrophic impairment in performing the TT, support the proposed association between caudate dysfunction and procedural learning impairment.

In the present study, the obvious weakness of all but the HDE 2 patients within the HD group on free recall (LP and BSRT), in the context of good recognition memory (DRT), is consistent with previous reports (Butters *et al.*, 1978, 1985;

Martone *et al.*, 1984). This could indicate, as Butters and his colleagues have suggested, that normal search strategies cannot be initiated in HD. It is also possible that free recall, being the more effortful and complex declarative operation, is at greater risk than recognition memory (Butters *et al.*, 1986).

In a large group of PD patients, better representing the full range of severity, we have documented an impairment in a small cluster of cognitive functions thought to depend primarily on the status of the prefrontal cortex (Taylor *et al.*, 1986). Adopting a functional/anatomical hypothesis, findings were interpreted as evidence that transmission of error signals, originating in the caudate and fed back to the dorsolateral cortex by thalamic relay, affect certain frontal-sensitive functions selectively. In this regard it was notable that PD patients could not perform novel problem-solving tasks efficiently unless provided with cues from the structured familiar knowledge base or explicit guidelines to follow. For example, unlike the amnestic patients in this study, they were inefficient in generating the mental strategies necessary to solve the Wisconsin Card Sorting Test (Taylor *et al.*, 1986).

A critical question then becomes whether the Tower of Toronto tests problem-solving rather than procedural learning and how these processes can be differentiated. We would argue that the basic ability of all but the HDA patients to perform the first phase of the test (TO) within normal limits attests to a fundamental preservation of problem-solving ability on the tower paradigm. A second consideration is equally germane. In TT1, there is greater variability of performance as compared with TT2, even within NC groups, the higher variance across the first 5 trials being due to the vicissitudes of hypothesis-testing and the search for successful heuristic strategies. Nevertheless, in TT1, the PD patients' trial-by-trial group scores remained within 1 SD of those achieved by their matched controls. Such performance failed to indicate significantly poorer PD related problem-solving within this phase of the test. It is only in the TT2 phase that meaningful comparisons in terms of learning can be made between groups. The data indicate that the PD patients continued the same general pattern of performance on the second block as they had shown in the first. In contrast, their matched controls progressively improved during TT2, approaching criterion in the last 3 trials, a trend which stabilized by the end of the test. This was clear in terms of both score achieved, and a shrinkage in SD units to 3 on trial 10 as compared with between 9 and 12 during the first 7 trials. At this point (trial 10) the PD score of 26 was, itself, 3 SDs above the NC group. The latter portion of the exercise (trials 8–10) clearly represents the learning as opposed to the problem solving component of the test and reflects a failure in the PD group to profit from continued practice.

In contrast to the parkinsonians, the HDE 2 patients lost a great deal during the 1.5 h gap between TT1 and TT2 and were slow to resume their course of improvement during the second block. The PD, HDE 1 and amnestic patient groups all maintained acquired proficiency during the TT1 to TT2 delay which can be taken as an indication of preserved savings. However, the PD patients showed little evidence of further improvement during TT2. Therefore, the PD and HDE 2 patients demonstrate a true procedural learning impairment in the presence of preserved problem-solving skills and adequate declarative memory.

The question then arises as to whether neostriatal dysfunction is uniquely responsible for the observed impairment in procedural learning as measured by the Tower of Toronto task in the early PD and HDE 2 groups. We have previously argued (Taylor *et al.*, 1986) that the best explanation for observed cognitive impairments in nondemented PD patients involves consideration of the so-called 'complex loop' involving prefrontal-caudate circuits (DeLong *et al.*, 1983; Alexander *et al.*, 1986). With regard to cognitive or nonmotor domains, influences arising from widespread regions of the cortex can be seen as funnelling through the caudate nucleus to substantia nigra reticulata (and a restricted portion of globus pallidus) which, in turn, project to ventralis anterior and dorsomedial (multiformis) thalamic nuclei finally to exert their influence on the prefrontal (especially dorsolateral) cortical region. Accordingly, the gradual acquisition of cognitive strategies, via experience with procedural tasks, would have to be established either in the nonmotor portions of the neostriatum (i.e., the caudate nucleus and the caudal-ventral putamen) or in the prefrontal cortex itself.

Preliminary studies at this centre in patients with documented damage in the prefrontal cortex support the suggestion that this portion of the 'complex loop' also plays a role in the Tower of Toronto solution. In 5 patients seen to date with subtotal removal of prefrontal cortex to excise tumours (3 right frontal lobe, 2 left), scores obtained on TT2 significantly exceeded those of the large NC group (Saint-Cyr *et al.*, 1986; and unpublished observations). Moreover, in a recent study of patients with comparatively greater frontal cortical damage, serious impairment of the ability to solve an operationally even simpler 3 disc problem than the tower test (Tower of London) was observed while patients with posterior cortical damage performed significantly better (Shallice, 1982).

Accordingly, where frontal damage is extensive, it appears that the hypothesis testing and planning which permits solution of a 3 disc problem, in which the number of moves does not exceed attention span, becomes unavailable. The PD, amnestic, HDE and NC all had no difficulty with this stage. NC, amnestic and HDE 1 patients subsequently solved TT1 and TT2 but at a cognitive level which could not be encoded and placed within the declarative system following 10 trials. In contrast, the PD, HDE 2 and HDA groups became overwhelmed once the number of moves exceeded their ability to 'hold the solution' in attention span during visual inspection.

Our current understanding of the mechanisms underlying the motor symptoms in PD, in which the automatic execution of learned motor programs is lost (Marsden, 1982), emphasizes the influence of disturbed putaminal outflow on the supplementary motor cortex which ultimately relays error messages to the primary motor cortex (*see* DeLong *et al.*, 1983; Alexander *et al.*, 1986). Pertinent to our understanding of the role of neostriatal outflow circuits on simpler types of procedural learning is the consideration of oculomotor and visuomotor functions. These would primarily be involved in tasks such as mirror image reading and the pursuit rotor. Indeed, to date, certain oculomotor deficits in PD, such as reduced gain of the vestibulo-ocular reflex and impaired saccadic control, are best understood by consideration of the impact SNr-relayed dysfunction exerts upon the superior colliculus (White *et al.*, 1983*a*, *b*). The two major neostriatal efferent pathways lead to the prefrontal cortex and the superior colliculus. In the oculomotor domain, it has been demonstrated that combined destruction of both routes is necessary for the abolition of voluntary oculomotor control (Schiller *et al.*, 1980). A similar set of lesions might be necessary completely to disrupt all cognitive and motor skill procedural learning tasks. The performance of patients with basal ganglia disease on cognitive procedural tasks suggests that frontocaudate circuits may be critical for the heuristic search for successful procedural strategies which, when established, fall under the dominion of frontal and allied cortical regions.

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APPENDIX: THE TOWER OF TORONTO TEST

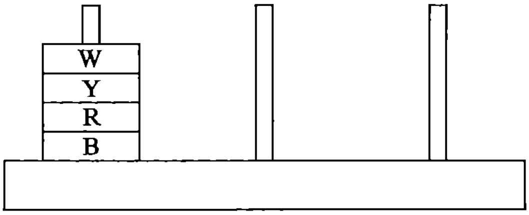

FIG. The base of the stand is 27 cm × 10 cm and is 2.5 cm thick. This is made of wood or plexiglas. The pegs are 0.9 cm in diameter, 8 cm high and 9 cm apart. The discs are 1.5 cm thick and 5 cm in diameter with a hole diameter of 1 cm to permit easy sliding onto the pegs. The discs are colour-coded: W = white, Y = yellow, R = red, B = black.

Setup

Beside the peg stand (*see* fig.) are two large colour photographs showing the start and finish positions clearly labelled. Also the two rules are written out and the colour code indicating darkest to lightest disc is provided on a separate card. These photographs and instructions remain in full view throughout the test and may be propped up

Instructions for Tower of Toronto 3 disc phase (TO)

'This is a puzzle I want you to try. The point is to move the stack of discs from this peg (pointing to leftmost) to this other one (pointing to rightmost) as shown in these photographs. You can put a disc on any one of the three pegs.

'There are only two rules. (1) You can only move one disc at a time. (2) You can never place a darker disc on top of a lighter one. For example (pointing to discs on stand), red can never be put on top of yellow and black cannot go on top of red or yellow.

'To start with we will use only 3 discs, the yellow, red and black. Remember the two rules, try to plan ahead and take your time '

Three trials are given.

Instructions for Tower of Toronto 4 disc phases (TT1, TT2)

'Now this is the same puzzle but we add a white disc on top The same two rules apply and no disc can be put on top of the white one.'

Five trials are given (TT1). Then there is a 1.5 h hiatus and 5 more trials (TT2) are given. After the last trial of TT2, the subjects are asked if they can explain how they did the task (i e., what is the successful strategy). E.g., 'Can you explain to me how you solved the task? Which steps are the most important? How would you teach someone else to do this?' With only 10 trials' experience, no subjects have ever been able to articulate what was their strategy.

General

If a subject cannot solve the 3 disc problem (TO) (i.e., 3 trials terminated at 25 moves or patient giving up) then there is no point going on to the 4 disc phase (TT). A partial demonstration may be given for certain patients (e.g., aphasic, deaf, foreign tongue-speakers) according to the judgement of the test administrator.

During the 1.5 h hiatus between TT1 and TT2 a variety of other testing is usually done This pause is to try to avoid the possible use of the recall of memorized sequences of moves as an aid to achieve solution.

If an error is made (attempted) such as putting a darker disc on top of a lighter one, the examiner says 'No, you can't do that move because of the rule. Put that disc back and try another move'. These errors should be noted because certain patients (e.g. schizophrenics, demented, large frontal lesions) may rule-break quite often and this would be significant. These errors (or illegal moves) do not count in the final tally since they are effectively cancelled by the test administrator.

If a subject picks up a disc and, without letting go, tries it on more than one peg, this counts as only one move. However, this tactic is discouraged. If the disc is let go and the subject changes his mind and wants to 'alter' the move, it is nevertheless counted.

Scoring

A simple tally of the number of moves (without counting illegal moves) per trial is tabulated. A ceiling of 25 moves per trial for TO and 50 moves per trial for TT is recommended since it restricts the administration time and prevents the patients from becoming too discouraged or tired.

A move-by-move notation may be used (e.g., Y2, R3, Y3, B2, etc., indicating the peg upon which each disc is placed in sequence) This could then be used to plot the choice path (*see* Cohen *et al*, 1985).

Calculation of the Index Score

For the 10 trials of TT1 and TT2, the number of trials requiring only 17 moves or better is counted (N1). From this total, the number of trials at 30 or more moves is subtracted (N2) In order to keep all Index Scores greater than 0, 10 is added.

i.e., $(N1-N2+10) = \text{Index Score}$

Our experience indicates that performance can be considered impaired if the Index Score is below 9

General Comments

Most young to middle-aged normal control subjects have an Index Score in the range of 13-16. Virtually all intact subjects can easily solve TO to criterion within 2-3 trials. In the first 5 trials of TT, it is not uncommon to see a great variability in score. It is in the second phase (TT2) that the number of moves per trial decreases most obviously, especially in the last 3 trials. If scores remain above 20 per trial by the end of TT2, an additional block of 5 trials could be given if desired to see if those subjects could eventually reach criterion levels of 17-15 moves per trial. In that case another 1 5 h pause between blocks would be advisable

These guidelines have been found useful in a clinical research setting and have proved suitable for a wide range of neurological and neurosurgical patients.

Brain (1988), **111**, 961-971

BOOK REVIEWS

Introduction to Clinical Neuroscience. By John Walton. Second Edition. 1987. Pp. 282. London: Bailliere Tindall. Price £12.95 (paperback)

The first edition of this book was published in 1983. The early appearance of a second edition is justified, the author believes, by fresh developments in neurophysiology, advances in the understanding of brain neurochemistry and changing neuroimaging techniques. Sir John has set himself an ambitious brief—to relate fundamental neurophysiological and neurochemical principles to normal and abnormal function in the nervous system. Indeed, he goes beyond this by including brief descriptions of both common and rare neurological diseases and neuroimaging techniques. Not only is the brief ambitious, but the intended audience catholic. Both the medical student and the practising neurologist or neurosurgeon are thought to be potential users of the book.

The number of chapters and their headings are unchanged from the first edition. The second chapter, entitled 'Some general principles of value in interpreting the pathophysiology of the nervous system' summarizes material appearing later in the text and might easily be eliminated. The author has been at pains to seek opinions from neurophysiologists regarding content but, despite this, parts remain at an elementary level which will hardly satisfy the neuroscientist. The description of normal and abnormal eye movement is a case in point. The concept of burst, tonic and pause neurons in relation to saccadic eye movement is not covered, despite its value in the better understanding of ocular motor disorders. Considerably more is known about the control of vertical saccades than the author's summary implies. There are other areas which suffer from over-simplification or from a failure to incorporate new material. The discussion of ascending pathways in the spinal cord fails to mention the spinocervical tract, or the fact that proprioceptive fibres in the gracile fasciculus leave it in the upper lumbar cord, which is occupied thereafter by fibres concerned only with superficial sensibility.

At times, there is a surprising failure to take advantage of topics which appear ideally suited to a discussion of basic mechanisms. Huntington's disease, for example, merits discussion of its genetic aspects, the evidence for the predominant involvement of the GABAergic system, and the role of PET scanning in establishing the presence of caudate hypometabolism in the early stages of the disease. Parkinson's and Alzheimer's disease would have benefited from a similar, multidisciplinary approach.

At the clinical level, several concepts are potentially misleading. The two principle causes of papilloedema are cited as increased intracranial pressure and optic neuritis. Atherosclerotic ischaemia of the optic chiasm is given as a cause of chiasmatic field defects, and the asymmetry of the hemianopia associated with optic tract lesions attributed to the nature of the causative compressive pathology. The concept of aberrant vessels compressing the trigeminal nerve as a cause of trigeminal neuralgia is described as recent, despite Dandy's contribution to the subject 60 years ago. Curiously, ventriculography and air encephalography are still described and illustrated.

In the final analysis, the aims of this text are too ambitious. An early initiative in the introduction of basic science as a means of enhancing the student's understanding of clinical neurology was made by Gordon Holmes in 1946. In his typically austere introduction, Holmes expressed a wish 'to explain the nature and significance of the symptoms and abnormal signs (with) which a patient with a nervous disorder may present'. There is little doubt that Sir John Walton's text will prove popular, and will proceed to further editions. At that time, the aims of the text should be reconsidered, and greater emphasis placed on basic mechanisms and their relationships to disease. Detailed descriptions of disease are out of place in a book of this nature, which should concentrate its attention on the medical student who wishes for a better understanding of how pathogenetic mechanisms operate in the nervous system.

G. D. PERKIN

What is Epilepsy? Edited by M. R. Trimble and E. H. Reynolds. 1986. Pp. 350. Edinburgh: Churchill Livingstone. Price £40.00.

In recent years, we have witnessed a remarkable proliferation of books on epilepsy leading to a certain repetitiousness. I was therefore delighted to find in this volume an original and personal approach to the problems of epilepsy often discussed informally but rarely addressed in print.

The book grew out of a workshop led by Trimble and Reynolds which attempted to probe the limits of epilepsy from a clinical, neurophysiological and psychiatric point of view. It reflects the interest of British neuropsychiatrists who have made and continue to make important contributions to our understanding of the borderland between epilepsy and psychogenic disorders.

The dean of British epileptologists, Maurice Parsonage, presents his critique of the International Classification; this kind of discussion is now rarely heard outside meetings of the International League Against Epilepsy. Some epileptic syndromes in infancy and childhood and their relevance to the definitions of epilepsy are discussed by Jean Aicardi The remainder of the first section of the book is devoted to differential diagnosis and to the age old question as to when a diagnosis of epilepsy is justified.

Information which can be obtained from recently developed monitoring techniques is presented by Wieser and Binnie. Their chapters introduce a group of contributions on the nature and significance of interictal epileptic EEG discharges.

The next group of papers on the borderland between epileptic and psychiatric disorders constitutes the core of the book. It reflects the views and perspective of the British School of Neuropsychiatry with papers by Fenwick, Toone, Fenton and Trimble and contributions from the US by Janice Stevens and Max Fink.

A rare opportunity is offered to get a glimpse of the early experiments with depth electrode recording in man carried out by Heath at Tulane. In a more orthodox vein, the next group of papers reviews experimental work on models of epilepsy, kindling, and the role of neurotransmitters.

The volume concludes with extensive discussions chaired by the editors. Printed discussions of this nature rarely read well nor do they achieve the exciting spontaneity of their live counterpart.

This is a stimulating and thought provoking volume which brings together reviews of findings and opinions on subjects to which international meetings usually devote little time. The experienced epileptologist will have little difficulty distinguishing opinion from fact in the nebulous borderland between epileptic and psychiatric dysfunction. The novice will be stimulated to reflect on these issues; he/she should guard against overenthusiastic speculation and should be encouraged to rigorously collect data instead. The type of data collection exemplified by the early depth electrode work is of course no longer possible and those studies, despite interesting findings, stand as a caveat.

Because of its unique and original approach, this volume should be recommended to all those interested in epilepsy.

F. ANDERMANN

The Management of Motor Neurone Disease. Edited by G. M. Cochrane. 1987. Pp. 104. Edinburgh: Churchill Livingstone. Price £4.95.

This short paperback was commissioned by the Motor Neurone Disease Association. The book is based on the experience of the Mary Marlborough Lodge unit at the Nuffield Orthopaedic Centre in Oxford—a unit well known to many neurologists for the excellence of its ministrations and advice for severely disabled patients. Dr Cochrane, the Medical Director of Mary Marlborough Lodge, two neurologists (Dr R. C. D. Greenhall and Dr R Langton Hewer), a speech therapist (Dr P. M. Enderby), and a physician in rheumatology and rehabilitation medicine (Dr B. P. Wordsworth) have contributed. Dr Cochrane says in his preface that the book is written for family doctors and primary carers but, as is pointed out in the first chapter, the prevalence of this disease is such that a GP on average will only occasionally look after a patient. It seems to me that hospital professionals who manage motor neuron disease would gain much from these pages.

Chapter 1 is a brief neurological description of the disease, its differential diagnosis and outline of medical treatment. Chapter 2 overlaps with Chapter 1 and there is a certain amount of repetition but some clinical illustrations are included. The meat of the book really starts in Chapter 3 on 'Communication and swallowing: problems and aids'. Detailed accounts are given about salivary dribbling, swallowing disorders, choking and speech problems, clinical problems which neurologists will recognize as among the hardest to cope with in this disorder. There are detailed paragraphs on the mechanism of each disability and a range of practical suggestions are given to alleviate the distress of each and improve function. I particularly liked the way that the authors encompassed a wide range of advice including tips for the patient to take up on their own at home as well as more technical matters such as when a palatal lift is indicated. Oddly, the book makes little reference to breathing disorders, although respiratory muscle weakness is inextricably linked to many of the bulbar problems encountered and results in specific symptomatology in some patients. Perhaps in a subsequent edition the question of assisted ventilation might be addressed.

The second half of the book is an account by Dr Cochrane of the wider management of patients. The first of these three chapters ('The early stages') starts with a brief discussion on 'Learning about the diagnosis'. This is a matter picked up by Sir John Walton in his Foreword to the book when he reflects on the change of attitudes in communicating this diagnosis. I suspect that many physicians would share the view expressed that '. . . the burden of truth lies with the physician who decides not to tell (the diagnosis)' once this is certain. Communication of this diagnosis, however, carries a responsibility for subsequent management—a responsibility not easily discharged with the scarce resources of manpower and ancillary services currently available. Questions of employment, benefit entitlement, mobility and an introduction to the relevant professional skills available for these patients follows. The next chapter details aids and appliances with constant emphasis on the team approach for prescribing these appropriately. Nutrition, mouth care, environmental control systems, mobile arm supports and manipulators are clearly dealt with. The last chapter discusses terminal care and bereavement and there are appendices about the Motor Neurone Disease Association and relevant addresses.

The level of clinical sensitivity in this book is high and few could be left complacent about their own practice having read it. As in many other chronic neurological disorders, the diagnostic process looks pretty easy when compared with making a good job of helping the patient—a task for which current neurological training is still insufficient. The main messages seem to be 'information' (for the patient) and a 'team approach', both based on adequate knowledge of the disease. This book is concise, sympathetic and useful and I would recommend it.

C. M. Wiles

Cerebrovascular Diseases. Proceedings of the 15th Princetown Conference on Cerebrovascular Disease. Edited by William J. Powers and Marcus E. Raichle. 1987. Pp. 374 New York: Raven Press. Price $118.00.

Proceedings of Conferences frequently make dissatisfying reading. They transmit little of the information that is actually presented as they are mostly written some months before the meeting itself and are often devoid of data and full of speculation. This book is entirely different. It has been imaginatively edited into groups of topics of immediate interest to cerebrovascular disease. Each topic comprises 5 or 6 papers often presented in mildly adversarial fashion and with an introductory comment by one of the editors or summaries presented in the form of discussions.

The contribution of the basic sciences to research in cerebrovascular disorders is rapidly increasing. The topic of cerebral protectors, currently focusing on antagonists of excitatory amino acids, particularly at the NMDA site, is stimulating world-wide interest. There appears to be a reasonable hypothesis to explain late ischaemic cell death and a means now of examining it. This section is well covered and the controversy surrounding the use of such agents in various animal models of ischaemia is well exposed.

Another topic of considerable interest at a time of expanding investigations into cerebral ischaemia with positron tomographic techniques is that of the coupling of cerebral perfusion to metabolism. Studies in pathology, and more recently with physiological stimulation of the normal brain, suggest that not only may perfusion and oxidative metabolism uncouple but there may also be similar decoupling of oxygen and glucose metabolism. The mechanisms underlying these derangements of metabolism and control of perfusion are also being investigated by techniques involving examination of the innervation of blood vessels at ultrastructural and neurochemical levels.

For the clinician there are also groups of papers on recent thoughts concerning practical methods of management and treatment. The carotid endarterectomy controversy is aired again and further information is provided regarding haemodilution in the acute phases of cerebral ischaemia. There is also an interesting paper from Miami which addresses the question of recovery of function following ischaemia by experiments performed in an animal model.

It is difficult to find areas of the book which are less than satisfactory. I think this reflects a considerable involvement of the editors in the preparation of the volume. The papers themselves are short, terse and well referenced. The subjects are well chosen and reflect the current increase in investigative activity at the basic and clinical level in cerebral ischaemia. It is to be recommended to anyone working in the field and to those wishing to bring themselves up-to-date with recent advances in the subject.

R. S. J. FRACKOWIAK

Head Injury. Second edition. Edited by Paul R. Cooper. 1987. Pp. 498. London: William and Wilkins. Price £75 00.

Although the UK is a relatively safe place in which to live, with deaths from road traffic accidents, particularly head injuries, approximately half those of equivalent European countries, there is a feeling that avoidable deaths still occur Attempts to reduce this mortality, often in young and productive people, focus on the use of high technology intensive medical management of the more severely injured, and in this country, on the identification of the patients judged to be at risk of secondary deterioration for prospective neurosurgical assessment before deterioration occurs. This book is very much directed towards the first of these aims. It is a multiple author account of how patients with severe head injuries may be managed in neurosurgical departments and in the intensive care units. Medical management of raised intracranial pressure, including criteria for monitoring and the techniques involved, are extensively covered in a total of 80 pages and it is in these areas that the book excels. It is therefore a book for the neurosurgeon and for the specialist in intensive care and will have little practical value for junior medical staff in the accident and emergency department. Current controversies, such as the use of skull x-rays, are not considered in depth, and there will be little support in this country for the statement that the presence of a skull fracture rarely alters or influences the management of patients. Recently published criteria (*The Management of Acute Head Injury*, H.M. Stationery Office, 1983) on the indications for skull x-ray, admissions and CT scan are probably more relevant to the admitting general surgeon or doctor in the A and E department. The single sheet included in that publication gives all the information necessary to make decisions in these three critical areas. Indications for CT scanning are briefly considered in the book but few will agree that ventriculography should be performed if CT scanning is not available. The lack of guidance on CT scanning may be because in the United States any severely head-injured patient will rapidly come under specialist neurosurgical care. Certainly that is not necessarily the case in the UK and the identification of patients likely to show deterioration on the basis of the presence or absence of skull fracture, and the patient's state of consciousness at the time of examination, receives no mention in this book. Clearly neither approach should be exclusive and the way forward may well be on the basis of improved selection of cases for prospective CT scanning and the application of high technology management by committed and highly trained teams. As an exponent of the latter approach, this book is excellent. Perhaps future editions will include sections on patient selection, and on the place of intensive rehabilitation for brain-damaged survivors.

R. D. Illingworth

Postoperative Complications of Extracranial Neurological Surgery. Edited by N. H. Horwitz and H. V. Rizzol. 1987. Pp. 387 Baltimore: Williams and Wilkins. Price £67.00.

Books and even articles on the complications of neurosurgical operations are unusual. This seems surprising in view of the obvious hazards of surgery to the central nervous system and the recent rapid growth of negligence litigation against doctors. Perhaps it is a reflection of the widespread occurrence of the 'surgical amnesia' which may be necessary for the effective practice of neurosurgery.

This book is a companion volume to *Postoperative Complications of Intracranial Neurological Surgery* which was published in 1982 and, like its predecessor, is written by a number of experts from the United States and Canada. It should be a welcome addition to every neurosurgeon's library and indeed this reviewer can say that it is one of the most interesting and absorbing neurosurgical texts that he has read in recent years. It covers every type of mishap that may result from spinal surgery, including those procedures performed for pain control. In addition it deals with the complications of peripheral nerve surgery for injuries, tumours and entrapment neuropathies, and of carotid endarterectomy. A chapter on intracranial-extracranial bypass is based on the findings of the international cooperative trial, a study unique in that it led to the effective demise of a surgical procedure. A final chapter on radiological complications covers not only myelography but also conventional angiography and DSA and the general risks of radiation exposure. Full references are supplied at the end of each section.

Despite being a multiauthor book, this volume has few faults. It is well printed, well illustrated and well produced. The individual authors have researched their topics thoroughly, often exhaustively. Some of the chapters could perhaps have been broken down more radically into individual sections so as to make it easier for the reader to find specific topics, and in a few cases more tables would have been helpful. These, however, are minor criticisms, and the reviewer can firmly recommend this volume, not only as a source of reference but also as a stimulus to achieving higher standards of practice.

R. S. Maurice-Williams

Dementia. Medicine in Old Age. Edited by Brice Pitt. 1987. Pp. 346. Edinburgh: Churchill Livingstone. Price £40.00.

This book has provided a balanced perspective on dementia. Perhaps the title is somewhat misleading as the essential topic is senile dementia, Alzheimer type, with other causes of dementia appearing as relevant differentials and not explored in the same depth. With that in mind, however, there is something here for everyone involved either with clinical care of dementia sufferers or with dementia research. Neuroscientists should be stimulated by Colin Godber's spirited defence of old age psychiatry services and, I hope, affected by Alison Norman's plea for the carers of sufferers. Similarly the chapters on diagnosis, epidemiology, modern neuroimaging and drug treatment are particularly clearly written and therefore should be informative outside medicine.

This is a multiauthored text and perhaps the editor's hand could have been stronger at times to eliminate repetition. Thus history taking, examination and investigation of patients is covered by several authors in several chapters. The section on prognosis could easily be subsumed under clinical picture. Some important research studies tend to reappear several times, an example being the follow-up investigation of patients with presenile dementia by Maria Ron and colleagues. The results of this study appear no less than 5 times in 5 chapters and regrettably these quotes do not always agree on the basic data.

For a research worker, devoted now for some time to the field of dementia, it is very exciting to be able to say that a book published in 1987 is already outdated. The new discoveries in genetics are too recent to be included here. Presumably, therefore, this book will be revised from time to time. If so, there will be a chance to reduce some of the repetition and it would also be invaluable to have a chapter that gives criteria by which dementia research could be judged, given that research is reaching us from many disciplines, genetics, epidemiology, biochemistry and psychology, etc. It is very hard for a worker in any one of these fields to be sure of the quality of the findings from workers reported in other fields. Guidelines for everyone would be invaluable.

The final problem with the current book is its price. It seems a lot, which probably means that a copy will be lodged in departmental offices rather than be personally owned. If one were there, I would certainly recommend that a colleague embarking on research into dementia should read it.

ANTHONY MANN

Aging of the Brain and Alzheimer's Disease. *Progress in Brain Research*, Volume 70. Edited by D. F. Swaab, E. Fliers, M. Mirmiran, W. A. Van Gool and F. Van Haaren. 1986. Pp. 525. Amsterdam: Elsevier. Price Dfl. 350 00.

In recent years there has been a great proliferation of books about dementia, Alzheimer's disease in particular, and any addition to this long list needs justification. This book contains the proceedings of a summer school organized by the Netherlands Institute for Brain Research in 1985 and as in previous volumes in this series, the emphasis is on biological research rather than on clinical matters. To judge the value of the book one needs to turn to the two central sections. The first of these sections devoted to alterations in the brain includes some good reviews of the cholinergic system, monoamines and neuropeptides, the role of lipofuscin, dendritic proliferation and circadian rhythms in the ageing brain and Alzheimer's disease. The second, devoted to aetiological factors and animal models, discusses, yet again, among other topics, the role of aluminium and whether the pathology of the nucleus basalis results from or causes the cortical abnormalities. There is also a review of the genetic basis of Alzheimer's disease, but the latest studies in this field are left out, as they would not have been available at the time of publication.

A smaller section on therapeutic strategies follows with a very good chapter on pharmacological aspects of muscarinic cholinergic agonists. The recent literature on THA must have arrived too late for inclusion, and some repetitive chapters on dietary manipulations make this section less interesting than the previous ones.

Most of the contributors are well known and perhaps unavoidably their contributions have the familiar flavour of something one has read before in other publications. The book is well presented and referenced with only a small amount of overlap between chapters. Two years ago this would have been an excellent up-to-date review of an important area; today that applies only to some of the chapters. Equally good reviews have appeared in other publications and given that some of the more recent information is not included in the book, it is hard to find convincing reasons why this book should be selected from among several others dealing with the same subject.

MARIA A. RON

Clinical Electromyography. By W. F. Brown and C. F. Bolton. 1987. Pp. 542. Guildford: Butterworth. Price £70.00.

Clinical Electromyography. By J A. R. Lenman and A. E. Ritchie. Fourth edition. 1987. Pp. 218. Edinburgh: Churchill Livingstone. Price £30.00.

The title is shared but the enormous differences in approach and content between these two books provide a vivid illustration of how the practice of electromyography has altered since the first edition of Lenman and Ritchie's work in 1970.

Brown and Bolton have edited a book which aims to marry the clinical analysis of neuromuscular diseases with the most appropriate electrophysiological techniques currently available, the basic premise being that electrophysiological studies are extensions of the clinical examination. I believe that to a large degree the authors have succeeded in their aim. The editors are from London, Ontario, and almost all the contributors are from Canada and the northern United States. Most are clinical neurologists as well as electromyographers and are prominent in the American Association of Electromyography and Electrodiagnosis. The chapter titles have been carefully chosen to reflect the clinical problems referred to the EMG laboratory and are thus a mixture of anatomy, pathology and physiology, rather than according rigidly to a single classification. A few examples provide the flavour of the book: Positive manifestations of nerve fiber dysfunction (Sivak and Ochoa); Radiculopathies and plexopathies (Eisen); Ulnar neuropathies (Miller); Inflammatory demyelinating polyradiculoneuropathy (Albers); Diabetic neuropathies (Wilbourn); Clinical neurophysiology of the spinal cord (Ashby). The book expects a certain working knowledge of electromyographic technique and is certainly not a 'methods' book—for that one must go to Kimura or one of the other standard works. Neverthless, many techniques are covered in considerable detail, especially various nerve conduction studies; there is much less on such electromyographic procedures as turns analysis. *Clinical Electromyography* is an invaluable source of information on the many questions which arise in the EMG laboratory: whether one needs to check up on the differences between the stiff man syndrome and neuromyotonia, the neuropathy of porphyria or lead poisoning, how to test conduction in the long thoracic nerve or what to expect in nemaline myopathy, the answers are readily available in this book, as well as detailed discussion of more routine problems. Certainly there are omissions but not too many: there is an inevitable overlap between the chapters so that what is missing on one can usually be traced elsewhere. For example there is no mention of X-linked bulbospinal muscular atrophy in 'Motor neuron disorders' (McComas), but it appears in 'Classification and electrodiagnosis of hereditary neuropathies (Thomas) (perhaps because it is better placed there?—Ed.). Given its world-wide prevalence it is perhaps surprising that there is no mention of leprosy. On a rather more esoteric note I must confess my regret that in the otherwise excellent final chapter, Mononeuropathies of the lower extremities, by Bolton, the closing paragraph neglects my favourite occupational palsy, genitofemoral neuropathy, due to the modelling of inordinately tight jeans. I do agree that there is no electrophysiological test for this!

This book, well produced and with remarkably few spelling mistakes, will be read with profit and enjoyment by any neurologist with an interest in the peripheral nervous system. It deserves a place in any EMG laboratory, close to the patients. I strongly recommend it.

Lenman and Ritchie's *Clinical Electromyography* is a very different work: the current fourth edition continues the pattern established in the first and indeed many chapters seem scarcely changed and are definitely now showing their age. There is still far more here on strength-duration curves than on the differentiation of axonal neuropathy from demyelination, for example. It remains a useful if somewhat stolid introduction to the field of electromyography, with a good bibliography of the most important early papers.

N. M. F. Murray

The Physiological and Technical Basis of Electromyography. By William F. Brown. 1984. Pp. 507. Stoneham, MA: Butterworth. Price £75.00.

Also from the London, Ontario, stable, this earlier publication is, in a sense, a companion volume to *Clinical Electromyography* by W. F. Brown and C. F. Bolton. Although devoted to the technical aspects and theoretical basis of electromyography and nerve conduction studies, it is written very much with the clinician in mind, not only for those who wish to undertake such procedures themselves, but also for those who refer patients for electrodiagnostic investigations. As emphasized in the Preface, neuromuscular disorders are common and, together, their prevalence exceeds that of even the most common of CNS disorders such as cerebrovascular disease. Our understanding of the pathophysiology of neuromuscular disorders has advanced very considerably during the past two decades or so and this book summarizes current knowledge in a readily understandable form. It is in no sense an instruction manual, for which the subject is now well served by a number of other publications.

The book begins, appropriately, with a short section on the limitations of electrodiagnostic studies, useful reading for clinicians who are not electromyographers but who refer patients for investigation. It emphasizes what information *cannot* be obtained by present procedures and some of the pitfalls of interpretation. Certain of the current 'blind spots' in electrodiagnosis are likely to recede with further technical advances. Thus the author mentions that the proximal portions of the peripheral nervous system are difficult to access. Since the book was written the use of stimulation of spinal motor roots using high voltage stimulators has been introduced and has gone some way towards improving this situation.

The full spectrum of electrodiagnostic studies is covered, including peripheral nerve conduction, spinal and cerebral evoked potentials, muscle, and neuromuscular transmission. In each the physiological basis for normal activity is followed by a consideration of the abnormal physiology in disease states and how the findings are influenced by the recording techniques. Each chapter is well referenced, for those who wish to delve deeper.

P. K. Thomas

Electromyography in Clinical Practice. Second edition. By Michael J. Aminoff. Edinburgh: Churchill Livingstone. 1987. Pp. 362. Price £39.50.

The second edition of this concise and highly readable book is half as long again as the 1978 first edition, with four completely new chapters. The general lay-out is as before, with an introduction to basic concepts and chapters on nerve and muscle physiology, motor unit properties and electromyographic apparatus before the clinical aspects of electromyography and nerve conduction studies are dealt with. 'Nerve conduction studies: basic principles and pathologic correlations' is new, but self-explanatory, whereas 'Other electrodiagnostic techniques' describes briefly such diverse topics as quantitative aspects of EMG (including motor unit counting, SF EMG, macro-EMG and turns analysis), studies of nerve fibre distribution, blink reflexes, H and F responses and somatosensory evoked potentials. There is now a chapter on root and plexus lesions in addition to those dealing with upper and lower limb nerves and the facial nerve: all describe methods of testing and common clinical problems as well as providing normative data. The article on peripheral nerve disorders is sufficiently up to date (references to 1987) to include information on AIDS and Lyme disease among many others. The final chapter, after 'Neuromuscular transmission', is on aspects of the central and autonomic nervous systems and includes tremor, myoclonus and elements of autonomic testing. This is followed by a most helpful glossary of electromyographic terms.

Tables and illustrations are very good and the general presentation is notably clear. This book is eminently suitable as an introduction to electromyography for neurologists and clinical neurophysiologists.

N. M. F. MURRAY

Surgical Neuroangiography. Volume 2. **Endovascular Treatment of Craniofacial Lesions.** By P. Lasjaunias and A. Berenstein. 1987. Pp. 434. Berlin: Springer. Price DM 298.00.

This is one of a series of four volumes which, together, present a comprehensive account of the place and methods of embolization in neuroradiological lesions. This particular volume, which is self-sufficient, deals with the treatment of vascular malformations and tumours of the head and neck, excluding the brain substance. Both authors are neuroradiologists, but they are also physicians caring for patients with conditions which may benefit from embolotherapy, and they have gained extensive experience in this field. Both have made important advances, in particular, from the functional and neuroanatomical and the technical development aspects respectively.

The embolization techniques appropriate for each lesion and vascular territory are considered in detail, sufficient to commend the book to both prospective and experienced therapeutic angiographers. Extensive illustration with both clinical photographs and radiographs images, especially with beautiful subtraction angiograms, accompanied by labelled diagrams and detailed captions, add to its teaching value.

The clinical features, natural history, pathological aspects and the merits of alternative therapies for angiomas and vascular tumours of the head and neck are considered in sufficient detail and presented in perspective, which should make the book of value to all clinicians with an interest in this field.

BRIAN KENDALL

Clinical Imaging of the Cerebello-Pontine Angle. By A. Valvanis, O. Schubiger and T. P. Naidich. 1987. Pp. 202. Berlin: Springer. Price DM 198.00.

This book deals with the clinical radiological diagnosis of cerebellopontine angle lesions and, in particular, with differential diagnostic features. It is based on a large neurosurgical and otoneurosurgical experience obtained in the University Clinics of Zürich University, combined with an extensive review of the modern literature. Diagnostic emphasis is placed on computed tomography and magnetic resonance imaging, although the authors' experience of the latter technique at the time of writing is relatively limited. The various other diagnostic techniques used in the recent past are reviewed, mainly from an historical aspect, with the exception of angiography for elucidation of vascular lesions. In general, the authors make clear the distinction between useful and virtually obsolescent techniques, but reference to the use of metrizamide and cervical puncture for cisternography must be considered a minor oversight.

A very good brief description of the anatomy of the cerebellopontine angle, particularly in relation to CT, is high-lighted by good illustrations of anatomical dissections and cryomicrotome sections of the region.

Even though coverage is not comprehensive, all the pathological conditions which are likely to be encountered are very adequately described and illustrated. No new features are added to the literature, but the authors' material is used to supplement and endorse previous descriptions.

The book is written in good English and well produced, with excellent illustrations and a good index It is recommended to neuroscientists, radiologists and otologists interested in the imaging of angle lesions.

BRIAN KENDALL

Cognitive Neurochemistry. Edited by S. M. Stahl, S. D. Iversen and E. G. Goodman. 1987. Pp. 395. Oxford Science Publication. Price £30.00.

The chapters contained within this volume resulted from a symposium held in October 1986 to mark the opening of part of the Merck Sharp and Dohme Research Laboratories, Harlow, UK, the idea being to bring together those very different disciplines of cognitive psychology and neurochemistry in such a manner as to bridge the conceptual abyss which appears to separate them. This admirable concept is focused on the need for both disciplines in tackling the problems of cognitive changes such as occurs in Alzheimer's disease. Even so it was found necessary to divide the book into two distinct parts. The chapters of Part I deal with neuropsychological approaches to cognitive disorders, whereas the contents of Part II are concerned with neurochemical approaches to cognitive disorders. Perhaps never the twain shall meet.

The initial chapters of Part I are concerned with the assessment and characterization of cognitive function in dementia as it affects man. Morris and colleagues tackle the difficult question of neuropsychological deficits in Alzheimer's disease versus dementia in Parkinson's disease. Subsequently the behavioural psychologists detail the experimental approaches to cognitive function in dementia, specifically tackling the issues of amnesia, arousal, attention, emotion and personality. The chapter by Rupniak and Iversen illustrates the difficulties of producing a primate model of senile dementia but offers hope for the future. I particularly liked the chapter by Robbins and Everitt on psychopharmacological studies of arousal and attention. For me as a pharmacologist, this was the highlight of Part I, since in language I could understand, it puts together a neurochemical approach to behavioural psychology and as such fulfilled the objectives of the volume.

Part II of the book concentrates on two major issues. First, a pair of excellent chapters deal with the postmortem studies of neurochemical changes in cortical dementia and subcortical dementia. Subsequently, Eileen Joyce looks at neurochemical changes in Korsakoff's syndrome. Surely, it is from studies of this kind that major clues will arise to the type of pharmacological manipulations required to overcome alterations in cognitive function. Secondly, the very important issue of the involvement of altered cholinergic function in cognitive disorders is discussed. This is of critical importance since most experimental studies have centred on altered cholinergic activity in brain and alterations in memory and learning, while postmortem studies of the dementias tell us that changes occur in a variety of neurotransmitters. Dr Kopelman seems to consider that cholinergic depletion alone does not account for all the memory deficits of Alzheimer's disease or Korsakoff's syndrome. Altman and colleagues also suggest that cholinergic-based strategies alone may not provide the desired effect on cognitive function and review the alternative approaches available. However, Traub and Freedman remind us of the many advances being made in the design of novel cholinergic agents for muscarinic receptor subtypes. Perhaps this may provide at least a clear answer to whether acetylcholine is the be all and end all of cognitive disorders in man.

Cognitive Neurochemistry is a rational attempt to bring together the various approaches being undertaken to examine the basis and treatment of cognitive disorders in man. I found it a fascinating insight into this area and I would recommend it to all who are interested in the dementias and related disorders.

P. JENNER

Brain Peptides Update, Volume 1. Edited by J. B. Martin, M. J. Brownstein and D. T. Krieger. 1987. Pp. 261. Chichester and New York: John Wiley. Price £50.00.

Intended as a successor to the outstanding volume on *Brain Peptides* published in 1983, this book fails in its principal objective. It can hardly constitute an update, since most of the sixteen chapters do not cover papers which have appeared since 1984. A count on a randomly chosen article dealing with peptides in respiratory control showed that over half of the 82 references cited date from 1980 or earlier.

Presumably the familiar combination of late manuscripts and delays in printing have resulted in an overlong gestation. While this is of little consequence in some areas, rapidly expanding fields are severely disadvantaged and the chapter on receptors and second messengers suffers in this respect. Another flaw is the irritating lack of editorial consistency. Thus the references in some chapters fail to include last page numbers and it is impossible to differentiate between substantial papers and brief abstracts, which have in some cases been included. Also, there are considerable differences in the depth to which subjects have been treated and these do not correspond with the volume or importance of recent developments.

On the credit side, there are some excellent brief reviews, particularly those dealing with particular peptides or peptide families: the chapters on the thyrotrophin-releasing hormone, brain cholecystokinin, VIP gene expression and bombesin all provide concise, well-written updates. The chapters on methodological developments may be of less general interest, although there is an excellent chapter on in situ hybridization histochemistry and a lucid account of strategies in the structural analysis of brain peptides.

Overall, I reckon that this book is relatively poor value compared to its illustrious predecessor. The excellent up-to-the-minute reviews which appear in such journals as *Trends in Neuroscience* are stiff competition for anyone aiming to produce an update in hardback The editors of *Brain Peptides Update* clearly hope to produce future volumes: one can only hope that the blemishes apparent in this volume are corrected

JIM EDWARDSON

CORRECTION

In the article by K. Poeck and C. Luzzatti 'Slowly progressive aphasia in three patients. The problem of accompanying neuropsychological deficit' which was published in *Brain*, **111**, 151-168, 1988, the letters L and R were inadvertently reversed in fig. 4.

ANNOUNCEMENTS

First International Congress on Alzheimer's Disease

This will be held in Las Vegas, USA, on September 6–9, 1988, and will provide an interdisciplinary platform for the discussion of clinical, pathological, genetic, biochemical, laboratory diagnostic procedures and therapeutic strategies. Further information may be obtained from Medical and Scientific Conferences Ltd., 12 High Street, Wheathampstead, Hertfordshire AL4 8AA, UK. Telephone 058-283-4117/8.

18th International Epilepsy Congress

This will be held in New Delhi, India, on October 17–22, 1989. Further information may be obtained from Professor M. C. Maheshwari, Secretary-General-89, Department of Neurology, Neuroscience Centre, All India Institute of Medical Sciences, New Delhi 110029, India.

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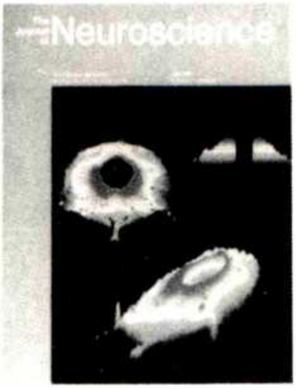

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The Official Journal of the Society for Neuroscience

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DALE PURVES, *Washington University, St. Louis*

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   (a) an abbreviated curriculum vitae and a list of any relevant publications,
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   (d) a recommendation from the head of department (where appropriate);
   (e) a letter of acceptance from the department to be visited;
   (f) a statement of costs likely to be incurred, and of other applications for funds that have been made. Advantage should always be taken of reduced air fares when available
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CONTROL OF ISOMETRIC FINGER FORCE IN PATIENTS WITH CEREBELLAR DISEASE

by NORBERT MAI,[1] PETER BOLSINGER,[1] MARC AVARELLO,[1]
HANS-CHRISTOPH DIENER[2] *and* JOHANNES DICHGANS[2]

(From the [1]Max Planck Institute for Psychiatry, München, and the [2]Department of Neurology, University of Tübingen, FRG)

SUMMARY

Control of isometric forces during grasping or handling of objects is an essential feature of all skilled manual performances. Previous studies of hand function in cerebellar patients were restricted to movements; force control was only investigated under isotonic conditions in combination with movements.

Control of isometric forces during voluntary contraction of finger muscles was investigated in 31 patients with chronic cerebellar disease and in 20 normal controls. Eight patients with Friedreich's ataxia were considered to be typical for a disease affecting the afferents to the cerebellum; 4 patients with anterior lobe atrophy, which affects leg movements to a greater extent than finger movements, were compared with 3 patients with hemisphere lesions; 16 patients suffered from diffuse cerebellar atrophy.

Using a recently developed microcomputer system for the continuous measurement of finger force, control of isometric force was studied in several tasks within the same subjects. The tasks included the maintenance of constant force output at different force levels with and without visual feedback, fast repetitive force changes, tracking of a sinusoidal target presented visually, and measurement of maximum grip force.

The amount of voluntary contraction in terms of muscular power was normal in all patients; in all other tasks clear deficits were observed. The extent of the disturbances was highly variable between patients even within the same clinical subgroup. The detailed analysis of force traces revealed a great variety in types of disturbance. The frequently observed slowing of the speed in repetitive force changes can be attributed to different components in different patients and not only to difficulties at turning points as might be predicted from comparable movement studies. In addition, performance deficits across different tasks were not uniform, indicating dissociation of impairment. Deficits in maintaining force, which may be interpreted as dysmetria or due to tremor, do not necessarily imply an abnormality in diadochokinesis, as measured in fast repetitive force changes, nor does the converse apply.

INTRODUCTION

The kymograph tracings of cerebellar deficits, which Gordon Holmes published in 1917, 1922 and 1939, have been reprinted in many reviews, even in the most recent, such as that by Gilman *et al.* (1981). This might be interpreted as respect for the excellence of Holmes' studies, but is more probably due to the fact that similar

Correspondence to: Dr Norbert Mai, Max Planck Institut für Psychiatrie, Kraepelinstrasse 2, 8000 München 40, FRG.

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studies are lacking. Holmes mainly reported the consequences of localized gunshot wounds of the cerebellar hemispheres, which are invariably more pronounced in the arm and hand than in the leg. While abnormalities of stance and gait as well as oculomotor symptoms due to cerebellar lesions have been investigated systematically (for reviews, *see* Dichgans, 1984; Dichgans and Diener, 1985), comparable studies on arm and hand function are still scanty. Among the rare examples of studies on patients are those of Terzuolo and Viviani (1974), Hallett *et al.* (1975) and Beppu *et al.* (1984), each concentrating on very specific aspects of disturbed movement.

The production of sufficient force and accurate control of force seem to be important aspects of all motor tasks in which a subject has to hold or handle objects between his fingers. Problems of force control were demonstrated in patients with cerebellar lesions by Holmes (1917, 1939). In acute lesions muscle strength may be reduced to half normal, and more persisting problems were found in calibration and maintenance of force (Holmes, 1939, fig. 9), in fast repetitive force changes (Holmes, 1917, fig. 7) or in matching of force between both hands (Holmes, 1939, fig. 5). All these tasks involved some type of movement, because the forces had to be exerted against strings whose strength was unfortunately not quantified.

Control of isometric forces has not been investigated in cerebellar patients despite the obvious fact that control of isometric force is also involved in holding or handling of objects (*see* Johansson and Westling, 1984). The investigation of the control of isometric forces, reported here, can be seen as an extension of Holmes' studies by the introduction of new tasks. We have developed a microcomputer system for the measurement of finger forces (Bolsinger and Mai, 1985) which was primarily designed for clinical use. By holding a force transducer between the thumb and index finger, isometric force can be measured continuously. Various tasks such as maintenance of constant force, fast repetitive force changes or force tracking are implemented as computer programs.

We present here data on various aspects of force control performance obtained in patients with cerebellar disease and in normal controls. The continuous registration of force traces enables a detailed analysis of the type of performance deficits. A concurrent recording of EMG data might seem desirable, but in the tasks used any evaluation of EMG data would be confronted with peculiar difficulties. For example, in maintaining a low isometric force between the thumb and index finger a cocontraction of nearly all of the many arm and hand muscles involved is to be expected (*see* Smith and Bourbonnais, 1981; and Discussion). This is quite different from analysing movements around a single joint which are operated by large muscles as, for example, during elbow movements. Irrespective of the possible contribution of EMG data, a disturbance of hand function has to be demonstrated first in terms of hand performance. The study of different force control tasks within the same person offers the possibility of analysing patterns of disturbances and, in particular, possible associations and dissociations between different aspects of force control.

METHODS

Subjects

Thirty-one patients with cerebellar disease (10 females, 21 males, aged 18 to 71 yrs, mean 40.5 yrs) and 20 normal controls (11 females, 9 males, aged 21 to 62 yrs, mean 40 yrs) were studied. All subjects were informed of the purpose and procedures of the investigation in advance and gave their informed consent. The clinical data for the patients are summarized in the Table. All patients suffered from chronic diseases and were seen within an ongoing clinical follow-up study. Eight patients with Friedreich's ataxia were considered to be typical for a disease affecting the afferents to the cerebellum. Sixteen patients were classified as having diffuse cerebellar atrophy, 4 as having anterior lobe atrophy and 3 patients showed hemisphere lesions. The patient group represents an ad hoc sample of chronic cerebellar patients with varying degrees of severity of disability. Diagnosis was mainly based on clinical data (most of the patients had been followed for many years in the Department of Neurology, University of Tübingen), as well as on CT scans and, in some cases, on neurosurgical reports.

Apparatus and methods

The details of the force measurement system used in this study were described in a recent paper (Bolsinger and Mai, 1985). In brief, the system consists of a modified Z80-based word processing system, 2 commercially available high precision force transducers (Interface Inc., Model SSM-AJ- 200N), a specially designed force signal amplifier with 2 identical channels for left/right hand comparisons and switch selectable force ranges of 5 N, 10 N, 25 N, 50 N, 100 N and 250 N and a menu-driven software package. The sampling rates can be increased up to 100 pps and amplitude resolution is 256 steps in each force range (for 5 N range this implies an amplitude resolution of approximately 0.02 N).

As a general procedure, the subject was seated in front of a monitor, which served as a display for task information (e.g. target force) and for the different types of feedback used in this study. The force transducer was held between thumb and index finger, all other fingers being flexed. During each measurement phase of 20 s duration, the transducer was held freely, the arm in a normal position at the shoulder and approximately 90 deg flexion at the elbow. Between measurements the hand holding the transducer rested on the table or the subject's leg. Task sequence was fixed for all subjects. Each task was tested for left and right hand, starting with the preferred hand or, in cases with unilateral deficits, always with the intact hand. All measurements reported below were taken in one session, which usually lasted 60 min, instruction and rest time included. Among the tasks implemented on the system, the following were tested on every subject.

1. *Maintenance of constant force* (*FHOLD*). The task was to generate a prehensile force (2.5 N) and to maintain this force constant for trial duration of 20 s. A vertical bar was presented on the monitor for visual feedback, its length corresponding to the actual force applied (continuous feedback). A horizontal line marked the required target force. The instruction was to align the top of the vertical bar to the target.

As a variation of this task, the visual feedback could be withdrawn and the force had to be held steady for an additional period of time. For this investigation we used the same prescribed force of 2.5 N and a withdrawal of visual feedback after 10 s. In addition, we tested the maintenance of constant force, using a target force of 12.5 N and continuous feedback available for trial duration. For each of the 3 conditions there have been 3 trials on each hand, not counting 1 or 2 trials to familiarize subjects with tasks.

2. *Fast repetitive force changes* (*FCHANGE*). The task was to produce, as rapidly as possible, force changes between 2 prescribed force levels (6.25 N and 18.75 N), which were presented again as horizontal lines on the screen. The actual force was displayed as a vertical bar. Two trials of 20 s duration for each hand were evaluated after 1 to 2 initial practice trials.

3. *Force tracking* (*FTRACK*). The task required the tracking of a sinusoidal target (frequency 0.1 Hz). The target (a vertical bar) moved between 2.5 and 7.5 N. Trial duration was 40 s, but only the

TABLE CLINICAL DATA

Case	Age (yrs)	Sex	Duration of illness (yrs)		Dysdiadochokinesia		Dysmetria		Intention tremor		Dysarthria	Oculomotor function	Ataxia of stance gait
					L	R	L	R	L	R			
Friedreich's ataxia													
1 (F01)	25	M	17		4	4	3	3	2	2	3	2	(5)*
2 (F02)	37	M	6		1	1	2	2	1	1	2	0	2
3 (F03)	18	F	4		2	2	2	2	2	2	3	2	3
4 (F04)	19	F	3		2	2	2	2	1	1	2	2	3
5 (F05)	20	M	13		3	3	4	4	4	4	4	3	(5)*
6 (F06)	27	M	19		2	2	3	3	3	3	2	3	5
7 (F07)	36	F	11		2	2	3	3	3	3	0	2	3
8 (F08)	24	F	10		2	2	2	2	3	3	0	2	3
Diffuse cerebellar atrophy													
9 (D01)	53	M	15		4	4	1	1	0	0	3	3	3
10 (D02)	20	M	14		3	2	3	2	0	0	1	2	4
11 (D03)	52	M	12		1	1	1	1	0	0	1	2	4
12 (D04)	71	M	5		0	0	1	1	0	0	3	3	3
13 (D05)	17	F	6		1	1	0	0	0	0	0	4	3
14 (D06)	48	F	20		3	3	2	2	1	1	2	3	3
15 (D07)	53	M	3		3	3	1	1	2	2	2	1	3
16 (D08)	38	M	6		2	2	1	1	0	0	2	2	3
17 (D09)	26	M	6		2	3	4	3	3	3	2	2	4
18 (D10)	32	F	2		0	0	1	1	1	1	2	3	3
19 (D11)	60	F	2		2	2	1	1	0	0	2	1	2
20 (D12)	64	F	3		1	1	3	3	3	3	2	3	2
21 (D13)	48	M	9		4	4	4	4	2	2	5	4	4
22 (D14)	54	F	2		4	4	3	3	3	3	4	3	4
23 (D15)	44	M	8		3	3	2	2	0	0	4	4	4
24 (D16)	63	M	6		3	3	3	3	1	1	5	4	4
Anterior lobe atrophy													
25 (A01)	44	M	10		0	0	0	0	0	0	0	0	2
26 (A02)	55	M	11		0	0	0	2	0	2	0	3	2
27 (A03)	51	M	4		0	0	2	2	1	1	0	3	3
28 (A04)	47	M	13		0	0	2	2	1	1	0	3	3
Lesion of cerebellar hemisphere													
29 (H01)	19	M	1	LH	3	0	2	0	3	0	1	3	1
30 (H02)	36	M	6	B	2	3	1	3	1	3	2	0	2
31 (H03)	56	M	3	B	2	0	3	2	3	2	0	0	2

L = left hand; R = right hand, LH = left hemisphere, B = bilateral; * = unable to stand. Rating of clinical symptoms: 0 = normal, no disturbance, 1–5 = mild to severe disturbance.

last 20 s were evaluated. The average rate of change (velocity) of target was 1 N/s, with a maximum velocity of 1.57 N/s. The actual force applied by the subject was displayed as a second vertical bar. The instruction was to hold both bars at the same height.

4. *Maximum grip force* (*FMAX*). The maximum grip force was measured only between the thumb and index finger, all other fingers being flexed. In 5 phases, each of 4 s duration, a subject had to press the transducer briefly and as strongly as possible. The start of each phase was announced by the experimenter and signalled visually by blanking the monitor screen. Rest time between contractions was variable, ranging from 5 to 10 s.

In addition to these tasks, all subjects performed at least one of the following additional tasks.

5. *Step mode tracking* (*FJUMP*). At the beginning of each of 6 trials, subjects were asked to produce and maintain a prehensile force of 5 N, indicated on the screen as a horizontal line. The actual force produced by a subject was fed back again using a moving vertical bar. After 10 s the target line jumped to a new position (2.5 or 7.5 N) not predictable by the subject. The instruction was to match the target as rapidly and as accurately as possible. In 6 trials we used 3 jumps to each position in randomized order.

6. *Maintenance of constant force with discrete feedback.* The task was essentially the same as the first: a force of 2.5 N had to be maintained for 20 s trial duration, but the type of feedback was different. Instead of the horizontal line signalling target force and the moving vertical bar, which indicated very precisely any deviation from target, only 3 states were signalled by the symbols: *, ↑, ↓. Whenever prehensile force was within a narrow tolerance range (defined as 2.5 N ± 0.1 N), a star was displayed on the screen. Forces outside this range were considered as error and indicated either by an up-arrow (more force to be exerted) or by a down-arrow (less force to be exerted).

In a subgroup of patients ($n = 10$), tested twice with about a 1 yr interval, performance data on both tasks (5, 6) are available together with retest results for the other tasks.

RESULTS

A typical record of normal performance is shown on the right half of fig. 1 from Case 29, whose left cerebellar hemisphere was nearly totally removed during operation for an astrocytoma. The data were recorded 11 months after surgery, from which he recovered quite quickly with surprisingly little performance deficit in the left hand. Maximum force (fig. 1G) was slightly reduced in the left hand (mean 84.0 N) compared with the right (104.5 N), but both values were within the normal range as was the difference between the 2 hands. The same was true for tracking performance (fig. 1F). The right side outperformed the left, but both performances were within the normal range. Fast repetitive force changes (fig. 1E) showed a reduction in rate on the left (45 changes in 20 s) when compared with the right (69 changes). The performance of the left hand fell outside the range of our normal controls, but regularity seemed quite similar in both hands. Maintenance of constant force was slightly disturbed in the left hand as compared with the normal performance on the right (fig. 1A–D). Using the evaluation standards discussed below, it can be shown that the performance on the left side is just outside the normal range.

Performance deficits in force control due to cerebellar disease were much more evident in 2 patients with diffuse cerebellar atrophy. Fig. 2 gives the results for a 54-yr-old female (Case 22) and fig. 3 data from a male patient (Case 24). Only maximum grip force was within the normal range for both patients; in all other tasks clear deficits were demonstrated. A different combination of disturbances is shown

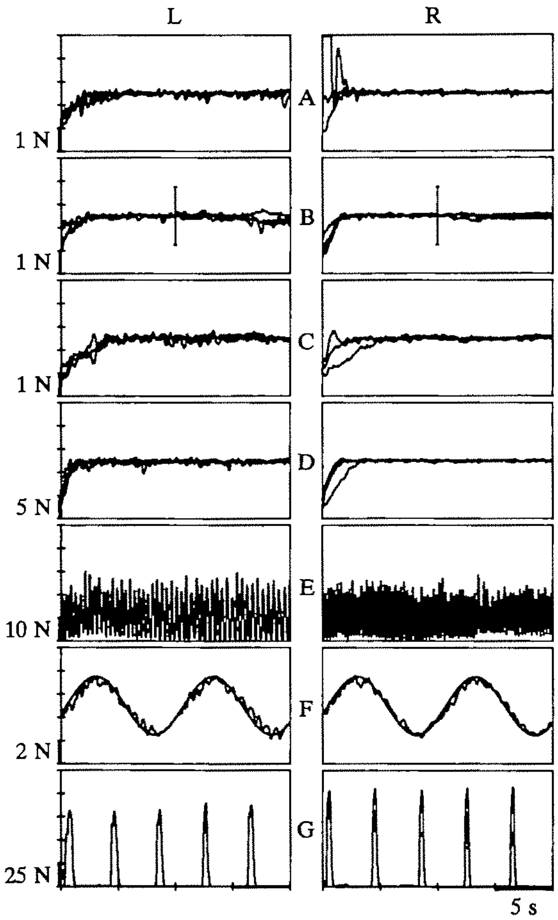

FIG. 1. Isometric force control in *Case 29* with a lesion of the left cerebellar hemisphere. L = left hand, R = right hand performance A–D = maintenance of constant force; 3 superimposed trials are shown for each condition. Target force was 2.5 N for A–C and 12.5 N for trial D. Target force was indicated by a horizontal line on a monitor; actual force exerted was fed back by the length of a vertical bar (continuous feedback) in A, B and D. The visual feedback was withdrawn in condition B for the last 10 s. In condition C a discrete feedback was used which signalled only one of three states ('correct force', 'too little' or 'too much' force) E = fast repetitive force changes; the changes required were between 6.25 N and 18.75 N. F = force tracking of a sinusoidal target. G = maximum grip force measured only between thumb and index finger (for further details, *see* text).

in the right half of fig. 4, where the maintenance of constant force is severely disturbed by tremor, but fast repetitive force changes are within the normal range. This performance was found in Case 28 with atrophy of anterior lobe of cerebellum, probably due to chronic alcohol abuse. For comparison, on the left half of fig. 4 performance in a normal subject is shown.

1. *Maintenance of constant force* (*FHOLD*)

Maintaining a constant force of 2.5 N for a trial duration of 20 s demands high precision, whereas the effort of producing the prescribed force seems to be negligible.

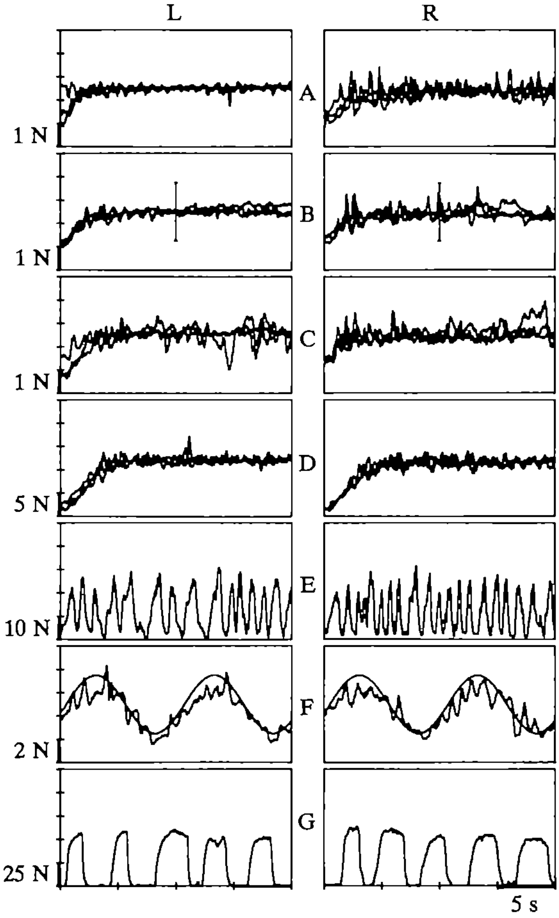

FIG. 2. Performance in *Case 22* with diffuse cerebellar atrophy. Same tasks as in fig. 1.

Normal subjects can maintain such a low force with great accuracy. As expected, performance is best under continuous feedback provided throughout the trial (Mai *et al.*, 1985). As a measure of performance we used the absolute error mean (AEM) calculated for the last 10 s of each trial. Error here means simply the difference between actual and prescribed force of 2.5 N. Given the experimental conditions used and an instruction which emphasized the accuracy of matching the target force, AEM is essentially a measure of variability. Taking this indicator, we found no left/right hand performance differences in the group of normal control subjects. Averaged across both hands, the AEMs varied between 0.0226 N and 0.0496 N, with a mean value of 0.0326 N across subjects. The overall mean and the very narrow range of performances (between 1 and 2% of target force) is quite similar to the results found in the normal sample investigated in our preliminary study (Mai *et al.*, 1985).

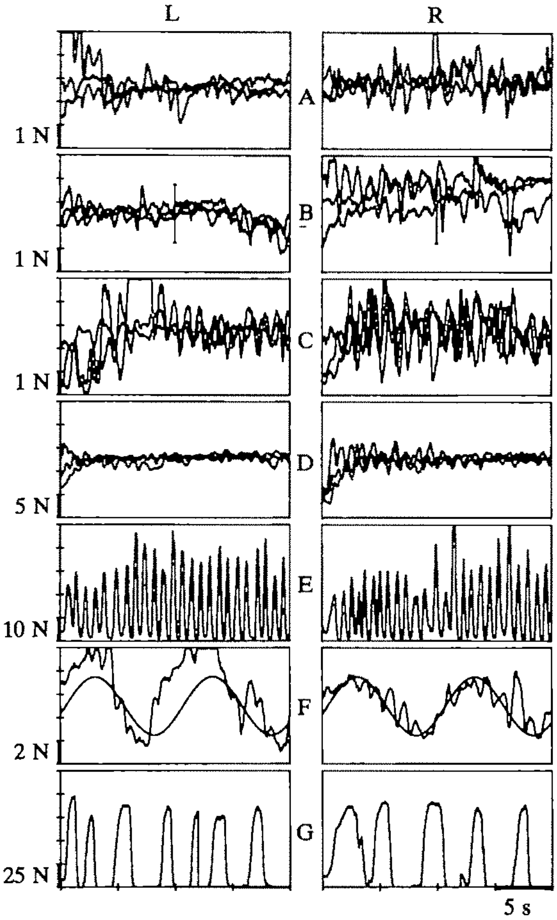

FIG 3. Performance in *Case 24* with diffuse cerebellar atrophy. Same tasks as in fig. 1

The range of normal performances in maintaining a prehensile force of 2.5 N, given continuous feedback, is shown in fig. 5 as the striped area. Compared with the limited variation in normal controls, there is a large variation between the cerebellar patients investigated. The most severe disturbances were found in patients with diffuse atrophy of cerebellum (Cases 23, 24). Disregarding these extremes, there is almost complete overlap between the groups. In each subgroup, patients with normal performance can be found as well as patients with varying degrees of deficit. Normal performance in this task does not always imply mild severity of clinical deficit, as can be seen in Cases 1 and 9. In general, none of the clinical symptoms listed in the Table can be taken as a sufficient predictor of performance in maintaining constant force. The highest correlation found is between 'oculomotor function' and right hand performance in maintaining constant force ($r = 0.45$). All other correlations are lower and none is significantly different from zero.

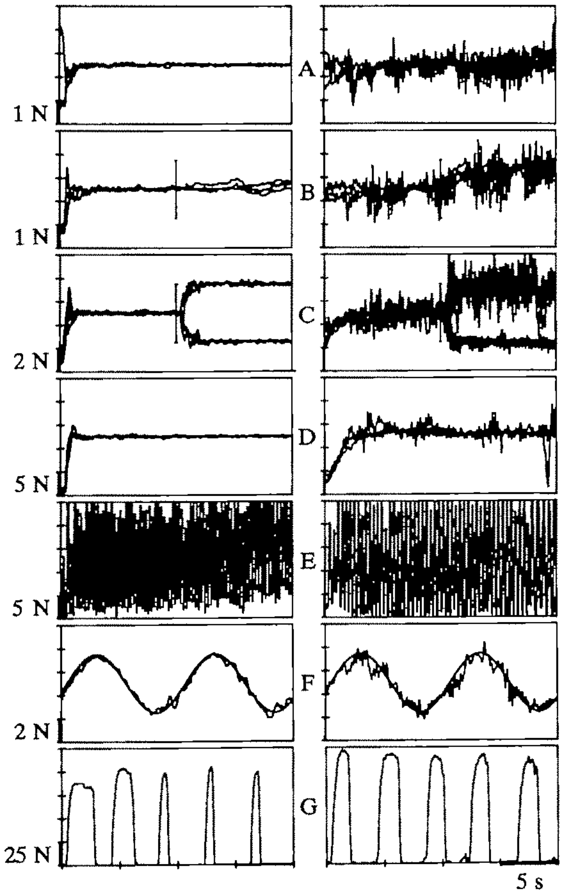

FIG 4. *Right*, performance in *Case 28* with atrophy of anterior lobe of cerebellum. *Left*, performance of a healthy control subject. Except for task C, all tasks are the same as in fig. 1. C, 6 superimposed trials of a step-tracking task in which target force jumped after 10 s from 5 N to 2.5 N or 7.5 N (for further details, *see* text). In task E the same performance as in E of figs 1-3 was required but the records were taken using an amplifier range of 25 N.

The individual force traces displayed in fig. 5 demonstrate the variation found in different types of disturbance. In some cases maintenance of constant force is hampered by sporadic tremor, as in Cases 17 or 28. In other patients corrective muscle contractions result in overshoot or undershoot (Case 20) which sometimes appear to be very brisk (Case 23). Another type is a sudden release of force (Cases 3, 21), which is immediately responded to by a corrective action. No uniform picture in fact emerged in any of the subgroups tested.

Pronounced left/right hand performance differences would be expected with unilateral lesions of cerebellum. Such a type of lesion is represented in our sample only by Case 29 (*see* fig. 1). In Cases 29 and 31 the lesions affect both cerebellar hemispheres. It is therefore not surprising that we found few differences between hands. The largest differences obtained are shown in figs 2 and 3. Case 24 (fig. 3)

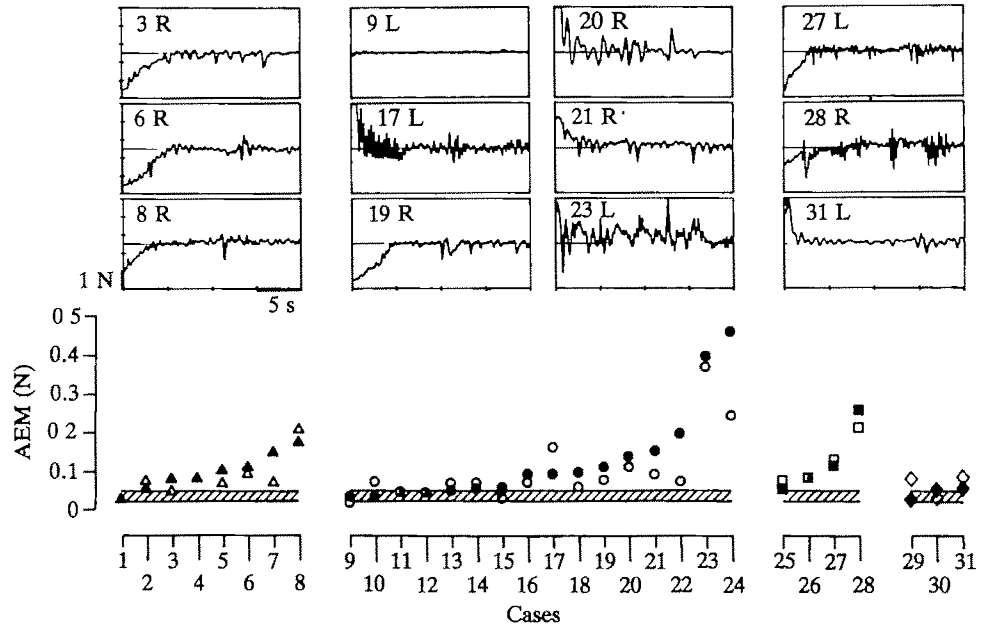

FIG. 5. Distribution of errors in maintaining a constant force of 2.5 N with continuous visual feedback. Absolute error means (AEM) were calculated for the last 10 s of each trial and averaged over 3 trials. The striped area indicates the range of values for normal subjects. Triangles = patients with Friedreich's ataxia; circles = patients with diffuse cerebellar atrophy; squares = patients with atrophy of anterior lobe of cerebellum; diamonds = patients with lesions of the cerebellar hemispheres. Open symbols = left hand; filled symbols = right hand performance. Patients were arbitrarily ranked by right hand performance. Examples of individual force traces are inserted.

presented extreme disturbances in both hands, and any calculated hand difference seems irrelevant. In Case 22 the left hand clearly outperforms the right in maintaining constant force under continuous feedback (fig. 2, task A), but taking into account the performance under the various experimental conditions (fig. 2, tasks B–D), the difference does not seem very definite.

Using 12.5 N as a target force for maintaining constant force output, the normal control subjects performed equally well as for the low prehensile force of 2.5 N. Again, performance variability across subjects was very low, and no left/right hand differences could be found. In terms of AEM calculated for the last 10 s and averaged across hands, the range of normal performance was found to be between 0.088 N and 0.211 N (mean 0.138 N). Relative to the force required, these figures are even slightly better than those for maintaining 2.5 N. Taking the AEM as a summary statistic, most cerebellar patients improved when maintaining 12.5 N instead of 2.5 N. One example can be seen in fig. 3 (task A vs D), where the AEM for the right hand (averaged over trials) is about 18% of target force of 2.5 N (fig. 3A) but only 5% of target force, when 12.5 N was required (fig. 3D). Nevertheless the 5% are clearly out of range of normal performance. Case 22 (fig. 2) is one of the rare examples where no improvement was seen when the higher force was requested.

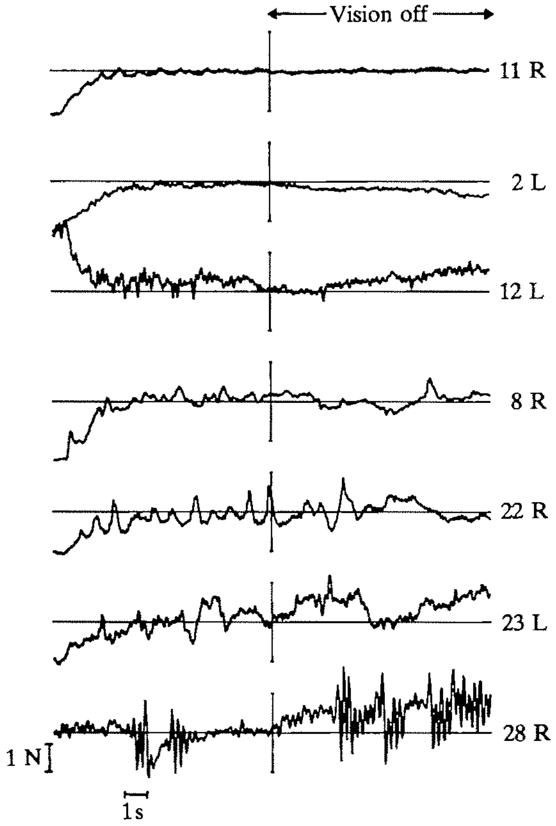

FIG. 6. Maintenance of constant force (2.5 N) with and without visual feedback. The force traces of *Cases 2* and *11* represent normal performance. Slight linear drifts were frequently observed in normal controls. All other force traces shown demonstrate various difficulties without vision which are not compensated under vision. At best, visual feedback helps to avoid some drifting but vision is ineffective in controlling the additional deficits, such as tremor in *Case 28*.

The control of constant force without visual feedback was investigated only for a target force of 2.5 N. Normal subjects were found to maintain low isometric force even under this condition with great accuracy (Mai *et al.*, 1985), a finding that was replicated in this study. In fig. 6 force traces of Cases 2 and 11 are examples of typical normal performances. While those for Case 11 are perfect, more usually slight linear drifts as in Case 2 or the right hand performance of Case 29 in fig. 1 were observed.

In cerebellar patients only slight performance differences under 'vision' as compared with 'no vision' were found. Using qualitative criteria (there is no adequate single indicator like AEM for drifting traces), 2 major groups can be distinguished. The first includes predominantly those patients who performed normally or nearly normally under visual control. Without vision, slight trends or minor irregularities, very similar to normal findings, were found. Patients in the

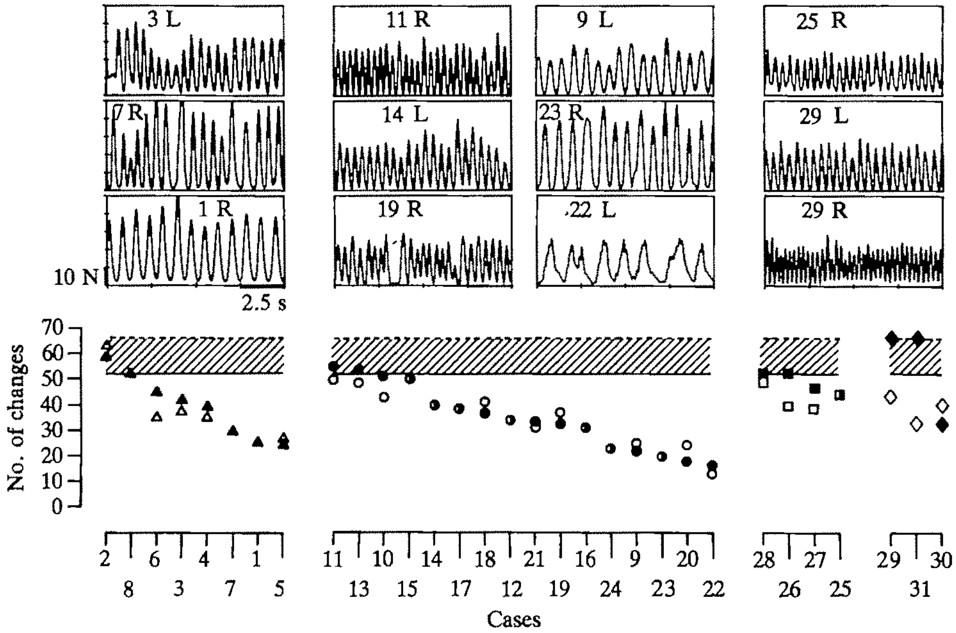

FIG. 7. Number of changes within 20 s. The striped area shows the variation of normal performance. Only the mean (broken line) and the lower bound (solid line) is shown. Patient data are ranked by right hand performance. All symbols for patient identification are the same as in fig. 5. The inserts show individual force traces for selected patients (for further details, *see* text).

second group demonstrated pathological performance without visual feedback, but performance was not greatly improved under vision. The force traces of Cases 8, 12, 22, 23 and 28 in fig. 6 are examples of this type of behaviour. The disturbances, which can be seen under the condition of 'no vision', seem quite comparable to those presented under visual feedback. In the sample investigated, no patients were found who, after withdrawal of visual feedback, showed the kind of decrease of performance usually seen in patients with severe loss of sensation.

2. *Fast repetitive force changes* (*FCHANGE*)

Taking the number of changes in 20 s as a rough estimate of individual speed, the normal control subjects varied between 52 and 85 changes. The mean across subjects was 66 changes. Differences between hands can be disregarded. The performance of cerebellar patients is summarized in fig. 7. Normal performance is presented as the striped area between the mean (broken line) and the lower limit in number of changes found in the control sample. Patients were ranked by right hand performance. The separation between normals and cerebellar patients was nearly perfect. Between the groups there was considerable overlap and the most severe disturbances were again found in patients with diffuse cerebellar lesions. As demonstrated by the inserts in fig. 7, the force traces show large variation between patients. The right hand

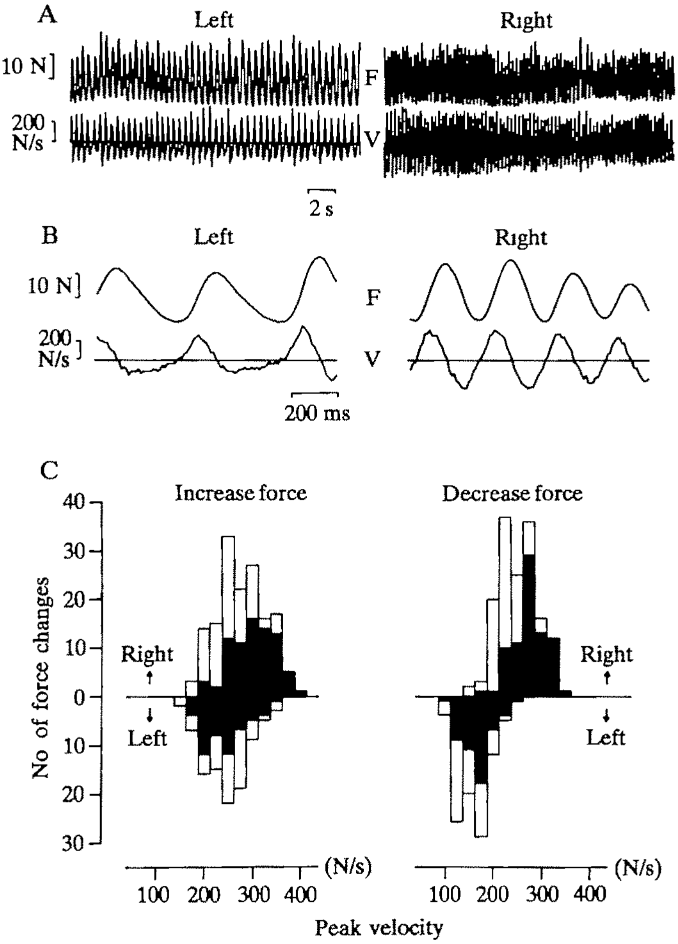

FIG. 8. Detailed analysis of repetitive force changes of *Case 29* with a lesion of the left cerebellar hemisphere (*see* fig 1). A, original force traces (F) for left and right hand and the corresponding derivatives (dF/dt), indicating the rate of force change or the 'velocity' (V). B, time sample of the traces shown in A C, histograms of peak velocities in 2 trials of 20 s duration. Filled bars indicate values from the first half of each trial, open bars values from the second half. The reduced speed in left hand performance is clearly due to difficulty in decreasing force.

performance of Case 29 (with a left cerebellar hemisphere lesion) corresponds to an average normal subject; the force trace of Case 11 R conforms to the lower limit of normal performance. Severe irregularities in both phases during increase and decrease of force were found in Case 22.

The reduction of speed in changing isometric forces seems to be a very general consequence of cerebellar disease. The continuous registration of force provides the possibility of further analysis of aspects which might contribute to the speed reduction. This is illustrated in fig. 8.

The analysis was undertaken for Case 29 (*see* fig. 1), which offered the unique chance of a direct comparison of left and right hand performance, since the cerebellar lesion affected exclusively the left cerebellar hemisphere. There is a clear reduction of number of force changes in the left hand (45) compared with the right hand (69). The amplitudes in the original force traces are quite similar, even a little smaller for the right hand (*see* fig. 8A, F traces). Differences in the rate of change of force (the first derivative of the force curve, velocity in N/s) therefore cannot be explained in terms of amplitude-speed relationship as discussed by Freund and Büdingen (1978). The corresponding velocity curves, shown as V traces in fig. 8A, demonstrate some slowing during the trial for the right hand but not for the left. The main effect is a clear reduction of velocity of the left hand compared with the right. This reduction is much more pronounced while decreasing force. This is documented by the histograms in fig. 8C representing the peak velocities over all trials. The overall

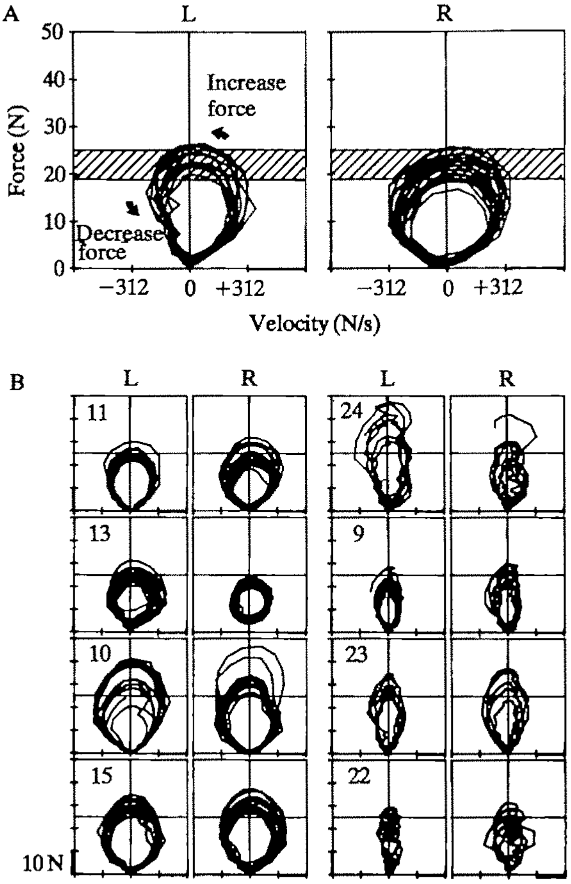

FIG. 9. Phase plane trajectories of fast repetitive force changes. Force and rate of change (dF/dt, 'Velocity') are plotted over time. Only corresponding time samples (5 s) are shown. A performance of *Case 29* (*see* fig. 8). The striped areas indicate the range between 18.75 N and 25 N within which the patient was asked to switch from increasing force to decreasing force. L = left hand performance, R = right hand performance. B, performance of selected patients (*see* fig 7) demonstrating the large variability between patients, even in the same diagnostic subgroup.

reduction of speed in the left hand performance of this patient can be attributed nearly exclusively to his difficulties in decreasing force. The relationship between force amplitude and rate of force change (velocity) can be further visualized by phase plane trajectories. Successive force values and their corresponding velocities are plotted as coordinates on a plane, the horizontal axis of which denotes velocity and the vertical axis force amplitude. In fig. 9A the results for Case 29 are shown. The figure contains corresponding time samples (5–10 s of second trial). Comparing left and right hand figures it can be seen that the increase of force and even the 'turning' between 20 and 25 N is quite similar in both hands. In decreasing force not only is peak velocity diminished but, in particular, velocity near the lower turning point.

This pattern of disturbances in fast repetitive isometric force changes due to a cerebellar hemisphere lesion seems surprising, at least in comparison with investigations of fast repetitive movements in cerebellar patients or animal studies. Holmes (1939) argued that 'the slower rate and the lack of rhythm depend mainly on time lost at the turn or in reversal' (p. 19). Conrad and Brooks (1974), using brief reversible cooling of the dentate nucleus in monkeys, provided evidence that the prolonged movement under cooling is due to problems at turning points for rapid alternating elbow movements.

For patients investigated, no general type of speed reduction in repetitive force changes emerged. This is demonstrated in fig. 9B. On the left, phase plane trajectories are shown for 4 patients whose performance is close to normal (*cf.* fig. 7). Such variation in peak velocity and force amplitude can also be found in normal subjects. This is contrasted on the right side of fig. 9B with the performance of 4 patients who are most disturbed in terms of the number of force changes per trial (*see* fig. 7). In Cases 9 and 23, reduction of speed might be attributed to problems at turning points as well as to a diminished peak velocity. In Cases 22 and 24 there is an additional disturbance by irregularities in force production and the velocity falls to zero randomly at each level of force amplitude. Between both extremes shown in fig. 9B, all intermediate forms of disturbance can be found.

3. *Force tracking* (*FTRACK*)

The right hand tracking record from Case 29 in fig. 1, as well as the record from Case 2 R in fig. 10, can be seen as representative of normal tracking performance. Such performance is achieved by normals without any specific or prolonged training. Only 1 or 2 practice trials have usually to be administered before actual measurement. In accordance with the performance in maintaining constant force, there are no consistent left/right hand differences, nor are there significant differences between sexes. As an overall measure for tracking quality the conventional root mean square error (RMS) (*see* Poulton, 1974) was calculated for the last 20 s of each trial. By squaring errors the RMS gives more weight to larger deviations of actual force from target force. The range of normal tracking performance, averaged across trials and hands, is shown in fig. 10 (bottom) as the striped area. Despite the heterogeneity of the sample of normal control subjects, the tracking performance

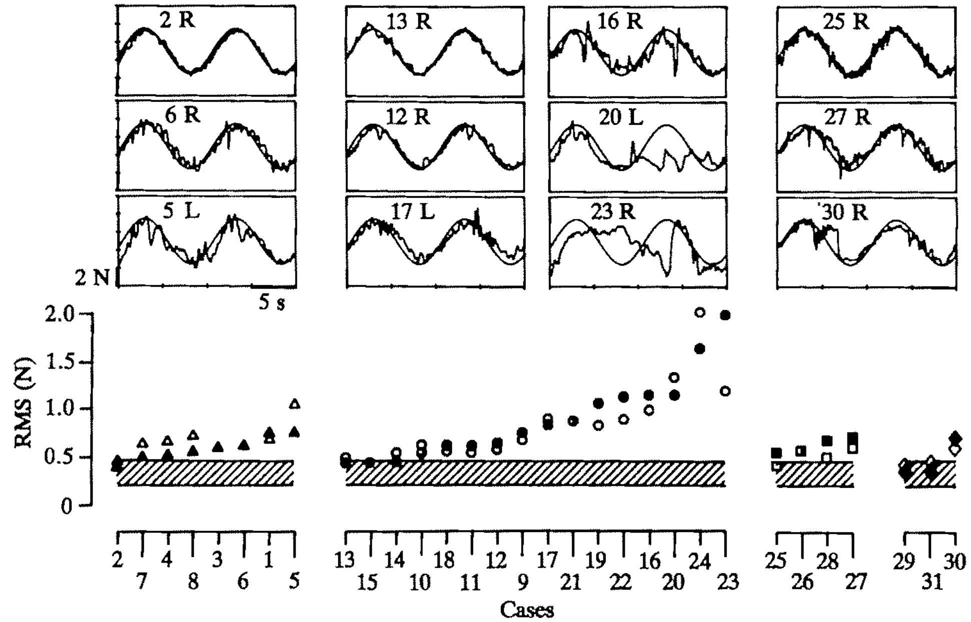

FIG 10 Performance of patients in a force tracking task. Performance was evaluated by calculating the root mean square error (RMS) for the last 20 s of each trial and averaging across the two 40 s trials. The range of normal performance is shown by the striped area. Performance of patients is indicated using the same symbols as in figs 5 and 7 Patients are ranked by their right hand performance. Examples of individual tracking performance are shown as inserts (for further details, *see* text).

varies only within narrow limits (RMS between 0.225 N and 0.460 N, mean 0.348 N). The tracking performance of the patient sample is shown at the bottom of fig. 10, again arbitrarily ranked by performance of the right hand.

The discrimination between patients and normal controls in tracking performance was nearly perfect, while there was considerable overlap between the different patient groups and, again, the most severe disturbances were found in the group of diffuse cerebellar atrophies. The phenomenological variation in types of disturbances is demonstrated by the inserts in fig. 10 (top). The records from Cases 17 L and 30 R show the irregular appearance of tremor, while performance in Case 25 R seems only slightly hampered by ongoing tremor. The tracking behaviour of Cases 5 L and 17 L is quite different, despite the similarity in terms of RMS. The same holds for the comparison of Cases 16 and 20, and in Case 23 R hardly any tracking can be detected.

4. *Maximum grip force* (*FMAX*)

As would be expected, there is a definite difference in maximum grip force between females and males. In addition, the variation within subgroups is considerable and hand differences are frequently observed. To obtain separate estimates for females and males, we pooled the current sample of normals with that of a previous study

(Mai *et al.*, 1985). The estimates of maximum grip force were based on 23 females and 21 males. For females the mean maximum grip force was found to be 63.5 N for the left and 69.0 N for the right hand. Irrespective of the hand used, the forces varied between 40 N and 98.6 N. The mean absolute difference between hands was found to be 9.9 N, the highest difference observed being 24.4 N. The corresponding figures for male normal controls were mean maximum forces of 94.4 N for the left and 98.9 N for the right hand. The forces were found to range between 61 N and 147.5 N. The average absolute difference between hands was 10.9 N, the maximal individual difference being as high as 37.5 N.

There was only one patient (Case 5) with severe Friedreich's ataxia, whose maximum grip force (L = 43.1 N, R = 50.8 N) was below the normal range for males. All other patients investigated produced forces within the respective normal ranges for females and males. The forces were evenly distributed over the whole range and therefore there was no way of separating patients from normals on the basis of maximum grip force. (All statistical comparisons of maximum grip force between patients and normals failed to reach statistical significance.) The reduction in muscle power of up to 50% due to cerebellar lesions, as reported by Holmes (1917), is a sign of acute lesions and is known to recover rapidly. Most patients investigated here are chronic cases (*see* Table) which may explain the apparent normality of muscle power.

5. *Relations between tasks*

It would be tempting to establish a definite order between the tasks investigated. For example, it might be asked whether the maintenance of constant force or the speed in repetitive force changes are more vulnerable following cerebellar lesions, which aspect of performance recovers earlier, or is a defect in one aspect always combined with deficiency in another.

Taking severity of disturbance as one aspect along which tasks can be ordered, an order relation between maximum grip force and all other tasks can be accepted. Nearly all patients investigated exhibited a grip force within normal range. In contrast, speed in repetitive force changes was found to be outside the normal range for most patients. Therefore, at least for this sample of patients, fast repetitive force changes can be said to be more disturbed than maximum grip force. A numerical comparison between the performance in both tasks could be achieved by calculation of z values, which relate the individual scores to the mean and SD of the control sample. A comparison between different tasks on the basis of z values or similar transformations (*see* Huber, 1973), while frequently judged as the 'via regia' for relating different aspects of performance, is restricted to very special cases. It has to be assumed that the SD are comparable estimates across the different aspects, although the performance variance can be widely manipulated by selection of task difficulty and by selection of control samples. For maximum grip force and fast repetitive force changes there were no restrictions for subjects to show their individual performance level. The tasks for maintaining constant force or tracking

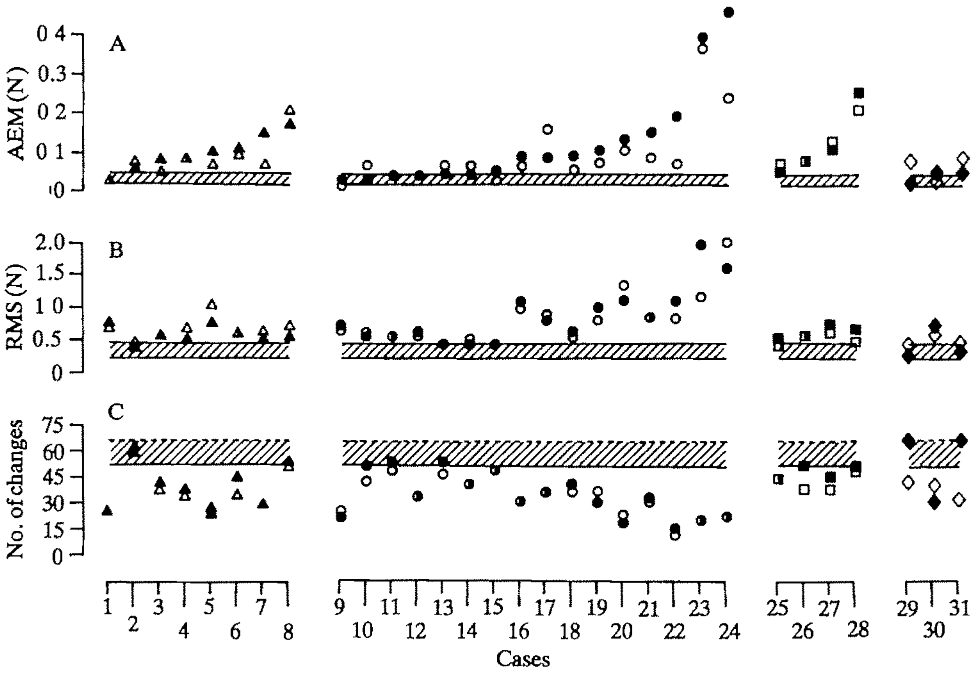

FIG. 11. Comparison of performance of cerebellar patients in different tasks of isometric force control. Replot of data from figs 5, 7 and 10, striped areas indicate the range of normal performance. For symbols, *see* fig. 5.

force were constructed so that they could be achieved by normal subjects without substantial training, whereby the true variability between subjects was probably artificially reduced. Based on artificially small SDs, z values would overestimate disturbances in patients as compared with tasks which allow a fair estimate of variability in population performance.

As might be expected, there was some correlation between performance scores for different tasks, as shown in fig. 11. In heterogeneous patient samples any measure of correlation would be dominated by only slightly disturbed patients (e.g., Cases 2, 10, 11, 25) and those which have severe deficits in every task (e.g., Cases 5, 20, 23, 24). To understand the relations between tasks it seems much more helpful to search for dissociations between performances.

Comparing the maintenance of constant force with fast repetitive force changes, obvious dissociations are easily found. For example, Case 1 was perfectly normal in maintaining constant force but his performance in fast force changes was quite impaired. The same relation held for Case 9 and just the opposite was seen in Cases 8 and 28. Such dissociations among task performance are a strong argument against the redundancy of tasks, which might be concluded from the overall correlation across patients. The fact that the performance in both tasks can be disturbed independently proves the functional independence of the behaviour tested. Similar dissociations were found when force tracking and maintenance of constant force were compared. Cases 1 and 9 show a pattern of performance directly opposite to the pattern in Cases 8 and 28. In comparing force tracking and fast repetitive force changes the dissociations found were much less (compare, e.g., Cases 8 and 9) and therefore caution might be exercised in their interpretation. It should be noticed, however, that for the group of patients with diffuse cerebellar lesions, hand differences occurred predominantly in holding force constant and in tracking, but not in the speed of force changes (*see* Cases 20–24, fig. 11).

In summary, the search for dissociations indicates the functional independence of tasks but fails to establish any clear order between aspects of performance. Data on the time course of disturbances, the reduction of deficits during recovery or under treatment, or the deterioration of symptoms with progressive disease, would be much more appropriate for assessing the vulnerability of the different aspect of performance investigated. Unfortunately, the repeated measurements sampled in this study are too small in number to allow any conclusions regarding this aspect.

The dissociations in task performance discussed are those that are most obvious.

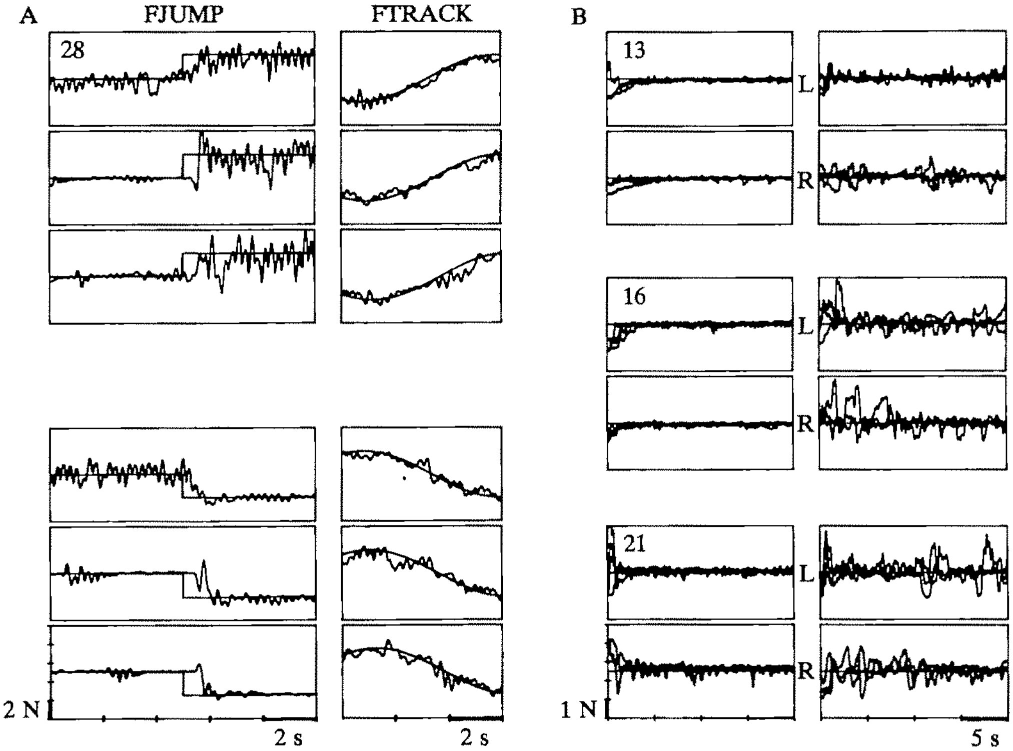

FIG. 12. Discrepancies in task performance due to variations in task characteristics. A, right hand performance of *Case 28* (*see* fig. 4) in step-mode tracking (FJUMP) and sinusoidal tracking (FTRACK). Performance is much better for steps from 5 N to 2.5 N (*bottom*) than for steps from 5 N to 7.5 N (*top*). Performance in sinusoidal tracking within the same force range is less disturbed than would be expected from deficits under hold conditions. B, examples of patients who show unusual deficits in maintaining constant force with discrete feedback (*right*) compared to continuous feedback (*left*).

Detailed analysis of task performance reveals many puzzling discrepancies, the understanding of which requires further detailed investigation. Some examples are given in fig. 12. In fig. 12A the adjustment of force to a sudden jump of target (step mode tracking) is compared with the adjustment of force in tracking a slowly moving target (ramp mode tracking). Data have been recorded for Case 28 (*see* fig. 4). The steps requested force 'jumps' from 5 N to 2.5 or 7.5 N, respectively. The target to be tracked also moved between 2.5 and 7.5 N. This patient exhibited great difficulties when asked to jump to the upper force level of 7.5 N. Force traces became very irregular immediately after the jump. Slow adjustment of force in the same force range did not produce any comparable irritation. In addition, jumps to the lower force level of 2.5 N resulted in a stabilized force trace which again is in sharp contrast to the patient's performance in holding constant a force of 2.5 N (FHOLD in fig. 4).

If only discrete feedback, instead of continuous feedback, is given for maintaining constant force (*see* Methods, Task 6), normal subjects usually produce slightly higher error values (Mai *et al.*, 1985). Normal subjects can hold constant force (2.5 N) under both feedback conditions fairly well and clear discrepancies between both types of feedback have been observed only in patients.

In fig. 12B examples of a clear decrease in performance due to a shift from continuous to discrete feedback are shown. All 3 patients maintained constant force under continuous feedback less well than normal controls (*see* fig. 5), but this cannot explain the decrease of performance under discrete feedback. Again there are obvious dissociations. Left hand performance under continuous feedback of Case 29 (*see* fig. 1) is quite comparable to the performance of Case 16, shown in fig. 12B, whereas the performance of both patients under discrete feedback is very different.

DISCUSSION

It can only be a matter of speculation as to why there have been so few attempts to replicate, extend or disprove the clinical observations of Gordon Holmes. Like other neurologists and psychologists during and after the First World War, he capitalized on the unique possibility to investigate the consequences of localized, in particular unilateral, brain lesions due to gunshot injuries. His investigations concentrated on the early and acute stages of injuries of the cerebellum. He defined his aim as 'to describe those disturbances of function which constitute the symptoms of recent cerebellar lesions as objectively as possible and to attempt to analyse complex symptoms into their simpler components' (Holmes, 1917, p. 463). Except for gunshot injuries, localized cerebellar lesions without additional involvement of the brainstem are hard to find. Gordon Holmes' evaluation of this situation needs little revision 70 years later, despite the revolutionary progress in brain imaging techniques. 'The opportunity of making uncomplicated clinical observations is rare in civil life, since acute lesions of the cerebellum, comparable with those produced by the physiologists, are uncommon; tumours and abscesses which develop in it are very liable to compress or influence the functions of other parts too; softenings and haemorrhages are rarely wholly limited to it, and the degenerative and atrophic diseases which involve it practically never affect it alone' (Holmes, 1917, p. 462). In routine clinical practice one is usually confronted with these 'complicated' cases. This might hamper any attribution of performance deficits to the site of the lesion but it does not imply that refined descriptions of deficits are not worthwhile.

As an extension of Holmes' studies we investigated the control of isometric finger forces in cerebellar patients. A cerebellar contribution to the control of isometric forces has not been demonstrated in clinical studies and only recently some neurophysiological evidence for cerebellar involvement was provided (Smith and Bourbonnais, 1981; Smith *et al.*, 1985).

The grip used in our investigation of cerebellar patients is very similar to that adopted by Smith and Bourbonnais (1981) in their monkey study; a force transducer has to be held between thumb and index finger. Smith and Bourbonnais recorded the activity of 27 individual arm and hand muscles which are involved in sustained prehension. The pattern of EMG activity revealed that virtually all antagonist pairs contract in approximate synchrony. This obvious cocontraction of antagonist muscles may help to stabilize the fingers at the carpal and metacarpophalangeal joints, and the actual finger force exerted results from a complex combination of agonist tensions and unequal tensions generated in the antagonists. Muir (1985) found a much more variable pattern of muscle activity in his investigation of precision grip as against power grip in humans as well as in monkeys. But he used a quite different manipulandum which involved certain movements of fingers and, probably more importantly, the force to be exerted was as high as 60 N in humans compared with the precise maintenance of low forces (1 or 2 N) required in the Smith and Bourbonnais (1981) study. In the various tasks we tested in the cerebellar patients, we used relatively low forces. In particular, holding constant a target force of 2.5 N required a high precision of finger force control. The probable cocontraction of antagonist pairs of muscles in isometric tasks has to be taken into account when our observations are compared with analysis of movement disturbances in cerebellar patients.

The maintenance of constant isometric forces was found to be very sensitive in separating cerebellar patients from normal controls. This might be taken as clinical evidence for a cerebellar contribution in performing these tasks. More direct evidence was provided by Smith and Bourbonnais (1981) and Smith *et al.* (1985) using single cell recordings in monkeys trained to maintain constant isometric force between the thumb and forefinger. The majority of Purkinje cells identified in the intermediate zone of the cerebellar cortex decreased their firing during the static phase in maintaining force (Smith and Bourbonnais, 1981). An increase of activity was found in the dentate and interpositus neurons during maintained grasping, predominantly in the static phase. A decrease in Purkinje cell activity and an increase of nuclear cell activity was to be expected since Purkinje cells are the major inhibitory input to the nuclear cells (Eccles *et al.*, 1967; Eccles, 1977). The overall discharge pattern of cerebellar neurons shows some similarity to the activity of neurons in the precentral cortex (Smith *et al.*, 1975; Cheney and Fetz, 1980), but is in contrast to the pattern for red nucleus neurons in a very similar task (Ghez and Vicario, 1978). In the red nucleus most neurons demonstrate a discharge pattern which is correlated with increasing force (the rate of force change) and not with the static phase in maintaining constant force.

This neurophysiological evidence of the contribution of the cerebellum in maintaining constant force may be seen as consistent with our clinical findings in cerebellar patients. Nevertheless, the types of deficit were found to be highly variable between patients even in the same diagnostic subgroup. At best, disturbances due to superimposed tremor may be separated out. This was observed frequently in patients with atrophy of the anterior lobe of cerebellum due to chronic alcohol abuse, but occasionally also in patients with diffuse atrophy or lesions of the cerebellar hemispheres. While tremor itself needs an explanation, for all other types of disturbances in maintaining grip forces, experimental data are lacking. At least it can be stated that other brain structures which have been demonstrated to be involved in maintaining constant force (Evarts, 1968; Cheney and Fetz, 1980; Evarts *et al.*, 1983; Jennings *et al.*, 1983) are insufficient to compensate for deficits in chronic cerebellar disease (*see* Sasaki and Gemba, 1985).

What is the role of visual feedback for the maintenance of low isometric grip force in cerebellar patients? Again, the variability of performance between patients does not allow an unequivocal answer. There was one major group of patients which performed normally or nearly normally under visual control. After withdrawal of visual feedback most of these patients demonstrated some drifts or slight irregularities quite similar to normal control subjects. The compensation for such drifts or irregularities under vision can be taken as an indicator for an effective use of visual feedback in these patients. The importance of visual feedback is underlined by those patients whose performance obviously deteriorated when, instead of continuous feedback, only discrete feedback was provided (*see* fig. 12B). Discrete feedback signals only 'correct force', 'too much' or 'too little' force, while continuous feedback informs about the precise actual deviation from target force. There was, however, another equally large group of patients who presented clear deficits in maintaining constant force without visual feedback which were not compensated by precise feedback (fig. 6). At least patients with these types of disturbances do not benefit from vision. This seems consistent with the frequent clinical observation that tests for dysmetria, like the finger/nose test, are usually performed no better with eyes open than with eyes closed. From monkey studies it is known that the cerebellum receives visual input from extrastriate visual areas relayed by corticopontine projections although the functional implications of these connections remain to be studied (for review, *see* Glickstein and May, 1982).

The role of the cerebellum in the control of fast repetitive movements has been discussed since Babinski (1899, 1902) first published his observations on diadochokinesis. The contribution of the cerebellar hemispheres to the initiation of rapid movements (Meyer-Lohmann *et al.*, 1977) and in the timing of agonist and antagonist activity in alternating movements (Conrad and Brooks, 1974) is now well established neurophysiologically and is consistent with clinical studies (Terzuolo and Viviani, 1974; Hallett *et al.*, 1975; Marsden *et al.*, 1977). In contrast, very little is known about cerebellar control of rapid isometric contractions (Meinck *et al.*, 1980). Based on the general idea that the cerebellum and, in particular, its lateral part, is primarily involved in the organization and timing of preprogrammed ballistic movements (Kornhuber, 1971; Allen and Tsukahara, 1974; Eccles, 1977), it can be expected that fast isometric contractions will be disturbed in cerebellar diseases. But dependent on the mechanical constraints, the necessary programs might differ for isometric and isotonic contractions. Fast repetitive force changes were found in this investigation on cerebellar patients to be a powerful discriminator between patients and normal controls. But further analysis of force traces revealed that between patients several factors contribute to overall slowness. A single cause of slowness, such as a delay at turning points comparable to those described by Conrad and Brooks (1974), can hardly be expected, given the variation of cerebellar lesions in the patient sample investigated. In addition, the sample included only 3 patients in whom the lesion involved primarily the cerebellar hemispheres. The variation of disturbances found corresponds to the variability of deficits reported by Hallett *et al.* (1975) for their (isotonic) elbow flexion task.

Discussion of the performance of cerebellar patients in isometric force tracking is again restricted by the fact that most studies on visuomotor tracking used some type of isotonic movement. A cerebellar contribution to the control of slow visually-guided movements was demonstrated by Mano and Yamamoto (1980) who recorded Purkinje cell activity during rapid and slow elbow tracking. Schieber and Thach (1985) recorded single-unit discharge in motor cortex, dorsal root ganglia and in dentate and interpositus cerebellar nuclei in Rhesus monkeys during a slow hold-ramp-hold tracking task. None of the neurons found in these nuclei demonstrated a clear relation to direction, position, velocity or load during tracking. Based on their data and on related results, these authors suggested 'that the dentate nucleus may help set levels of motor cortex, fusimotor, and spindle afferent activity . . .', while the 'interpositus may participate more directly in the detection of and the compensation for small movement irregularities and oscillations' (p. 1266). Tracking deficits during reversible inactivation of the cerebellum in monkeys were demonstrated by Brooks *et al.* (1973), Uno *et al.* (1973) and more recently for sinusoidal tracking by Miall *et al.* (1987). In cerebellar patients, deficits in tracking performance were reported by Beppu *et al.* (1984), who used the same slow tracking task as Mano and Yamamoto (1980). The isometric tracking task used in our study requires very precise control of low forces at a rather small rate of change. The results obtained with this task show a nearly perfect separation between cerebellar patients and normal controls. This might be taken as clinical evidence for a cerebellar contribution to visually-guided force tracking. Given the findings in maintenance of constant isometric forces, the results in force tracking might cause little surprise. Both tasks involve some tracking, either of a constant or a moving target, and performance in both might therefore rely on some error-correcting mechanism which uses both visual and proprioceptive feedback or an open-loop feedforward system. Since error-correction seems more necessary with moving targets, more problems would be predicted for cerebellar patients in tracking than in maintaining constant force. The finding that more patients performed within the normal range in maintaining constant force than in force tracking (*see* fig. 11) points in this direction, but there are clear exceptions (*see* Cases 8, 28, figs 11, 12A). In both cases force traces are more irregular in maintaining constant force than in following a moving target. One could speculate about this dissociation using the suggestions made by Schieber and Thach (1985), but it is beyond question that a better understanding of a cerebellar contribution to control of isometric force needs much further experimental study. For example, nothing can be said about the contribution of proprioceptive feedback in controlling isometric finger forces. Nagaoka and Tanaka (1981) have shown that proprioception or kinaesthesia is essential for smooth elbow tracking; visual information on arm movement alone is insufficient. It is at least questionable whether the same argument applies in isometric force tracking, since even normal subjects have great difficulties in matching isometric force between both hands.

Perhaps induced by Gordon Holmes' scepticism about any functional localization in the cerebellum, it is commonly believed that all symptoms of disordered voluntary movement occur together. While he studied larger samples (21 patients in 1917), he reported records of disturbed movements just as examples without relating these to individual cases. This presentation of data is in contrast to the report on hand sensibility by the same author (Head and Homes, 1911) and precludes any evaluation of coincidence between symptoms which is not explicitly discussed by Holmes. Perhaps this contributed to an overly uniform picture of cerebellar disturbances. However, for the control of isometric finger forces studied here, clear dissociations can be demonstrated. Deficits in maintaining constant force, which may be interpreted as dysmetria or due to tremor, do not necessarily imply an abnormality in diadochokinesis, as measured in fast repetitive force changes, and conversely. This is consistent with the conclusions of Hallett *et al.* (1975) derived from EMG analysis of elbow movements. While the available data are insufficient to discuss any relation of specific deficits to the site of lesions, the functional dissociations found should prompt further investigations.

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EVIDENCE FOR UNMYELINATED SENSORY FIBRES IN THE POSTERIOR COLUMNS IN MAN

by RUDY P. BRINER, SUSAN M. CARLTON,
RICHARD E. COGGESHALL *and* KYUNGSOON CHUNG

(*From the Marine Biomedical Institute and Departments of Neurosurgery, Anatomy and Neurosciences and Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas*)

SUMMARY

The human posterior columns are generally described as a myelinated fibre pathway. The present study demonstrates that more than 25% of the component axons are unmyelinated. Many of these unmyelinated axons are labelled by antibodies to calcitonin gene-related peptide (CGRP), a substance found in sensory cells, axons and terminals. On this basis we suggest that there are significant numbers of unmyelinated primary afferent axons in the human posterior columns. These results indicate that unmyelinated sensory axons are more widespread than previously thought, and that they should be taken into account when considering stimulation of the posterior columns to relieve pain.

INTRODUCTION

Mammalian primary afferent axons are subdivided into large diameter or coarse fibres which travel in the posterior columns (dorsal funiculi) of the spinal cord and fine fibres which travel in Lissauer's tract (dorsolateral fasciculus). Recent electron microscopic observations, however, show significant numbers of unmyelinated axons in rat and cat dorsal funiculi (Langford and Coggeshall, 1981; Chung *et al.*, 1985, 1987), and loss following dorsal rhizotomy shows that many are primary afferents (Chung and Coggeshall, 1985; Chung *et al.*, 1987). Thus it is important to examine the human posterior columns for unmyelinated sensory axons, particularly since posterior column stimulation is sometimes performed to relieve pain on the basis that only large sensory fibres will be stimulated (Nashold and Friedman, 1972; Murphy and Giles, 1987).

MATERIAL AND METHODS

The material that forms the basis of the present study was obtained from autopsies of adult humans of either sex, ranging in age from 24 to 80 yrs, who died from diseases unrelated to the spinal cord. Nine autopsies were done but in only 2 (24 and 25 yrs of age, death due to trauma and drowning) was fixation

Correspondence to. Dr Kyungsoon Chung, Marine Biomedical Institute, 200 University Boulevard, Galveston, Texas 77550-2772, USA.

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adequate. These were the 2 specimens where the time from death to fixation was 4 h or less. In most of our specimens fixation was 6–10 h after death and the material was unusable because of intracellular swelling. In each case the spinal canal was opened, and the caudal end of the spinal cord and attached roots were immersed in fixative consisting of 3% glutaraldehyde, 3% formaldehyde and 0.1% picric acid in 0 1 M, pH 7.4, cacodylate buffer. After 10–30 min the sacral spinal cord, which was well fixed, was removed and the caudal part of this block sliced into transverse sections approximately 0.5 mm thick. The constraints of the autopsy procedure prevented us from determining segment levels with exactness. Each section was placed in fresh fixative for 1–3 days.

The above sections were then trimmed into blocks of only the posterior columns, and the blocks were rinsed in 3 changes of 0.1 M, pH 7.4 cacodylate buffer. The blocks were then placed for 2 h in a mixture of 1% osmium tetroxide and 1.5% potassium ferricyanide (Langford and Coggeshall, 1980) in 0.1 M cacodylate buffer. This tissue was then rinsed in 2 changes of 0.1 M, pH 5.2 maleate buffer, placed in a 1% uranyl acetate solution in maleate buffer for 1 h, again rinsed in maleate buffer, dehydrated in ascending concentrations of ethyl alcohol, and embedded face down in a mixture of Epon and Araldite. Sections 1 μm in thickness were cut with glass knives and stained with 0.5% toluidine blue in a 1% sodium borate solution. Thin sections were cut and placed on single hole copper grids covered by a formvar film. The thin sections were stained in 0.2% lead citrate for 30 s, rinsed, dried and placed in a Philips 300 electron microscope.

Since the human posterior columns are so large, we restricted our examination to lower sacral segments and even here a sampling procedure was necessary. Our method, which has been used when estimating numbers of axons in peripheral nerves (e.g., Dyck *et al.*, 1968) was to determine numbers of axons per unit area in sample micrographs and then multiply by the total area of the column (from a low power montage of the dorsal funiculus taken from the same thin sections). As a formula: axon number (sample) × total area of the funiculus (montage)/sum of sample areas = total axon numbers in the funiculus at this level. The sample micrographs were located by placing a randomly orientated orthogonal grid over each montage. Each intersection of the lines of the grid was then marked on the montage and used as the central point of an electron micrograph taken at an original magnification of 3300.

Other thick (approx. 500 μm) sections fixed as above were stored in cacodylate buffer overnight. The next morning each of these was sectioned with a vibratome (Lancer series 1000) into 25 μm transverse sections. These were collected in 0.1 M phosphate buffer (pH 7.4). Each 25 μm section was placed in 1% sodium borohydride in buffer for 30 min and then rinsed in 6 changes of buffer for 5 min each. To aid the penetration of the antibody and still preserve the tissue for electron microscopy, each section was exposed to different concentrations of ethyl alcohol (10%, 25%, 40%, 25%, 10% in PB) for 5 min each. The sections were then stained with antiserum to calcitonin gene-related peptide (CGRP) (a gift from Dr Cary W Cooper, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas) by means of the peroxidase-antiperoxidase method (Sternberger, 1979). The antiserum was raised in rabbits against synthetic human α-CGRP coupled to bovine serum albumin and used at a dilution of 1:2000. When the CGRP antiserum was preabsorbed with purified CGRP antigen (50 μm/ml), there was no staining. Radioimmunoassay revealed less than 0.3% cross reactivity for human calcitonin and less than 0.001% for substance P, dynorphin, cholecystokinin, neurotensin, met- and leu-enkephalin, vasoactive intestinal peptide, bombesin, somatostatin and serotonin (Carlton *et al.*, 1987). Following the immunostaining, the tissue was placed in 1% osmium tetroxide in cacodylate buffer for 1 h, stained in block with 1% aqueous uranyl acetate, dehydrated in ethanol and flat embedded in a mixture of Epon and Araldite. Sectioning was performed as above.

RESULTS

The human posterior columns, as in other mammals, are the posterior spinal white matter bounded by the Lissauer's tract laterally, the median septum medially, the grey matter of the spinal cord anteriorly and the pial surface posteriorly. Easily visible cytological features of the posterior columns on light microscopy are myelinated axons, glial nuclei and blood vessels. On electron microscopy, these can be seen with greater clarity (figs 1, 2). In addition, unmyelinated axons can be seen in the interstices between the other structures (figs 1, 2). The unmyelinated axons are round or oval on cross section, contain neurofilaments and microtubules as well as occasional mitochondria and cisternae of the agranular endoplasmic reticulum, and range in size from approximately 0.1 to 1 μm in diameter (figs 1, 2). The unmyelinated axons can be distinguished from similar sized astrocytic processes because of the abundance of intermediate filaments and relative absence of microtubules in the latter (figs 1, 2). Typical desmosomes unite astrocytic processes in the human posterior columns (fig. 2). These are not seen in rats, and for this reason we presume they are related to the size of the organism.

A goal of the present study is to present counts of myelinated and unmyelinated axons from the human posterior columns, and the quality of fixation is an important issue in this regard. The fixation is not so critical for myelinated axons because they possess an identifying sheath, but it is impossible to identify unmyelinated axons with certainty in poorly fixed material. This is because glial processes and axons

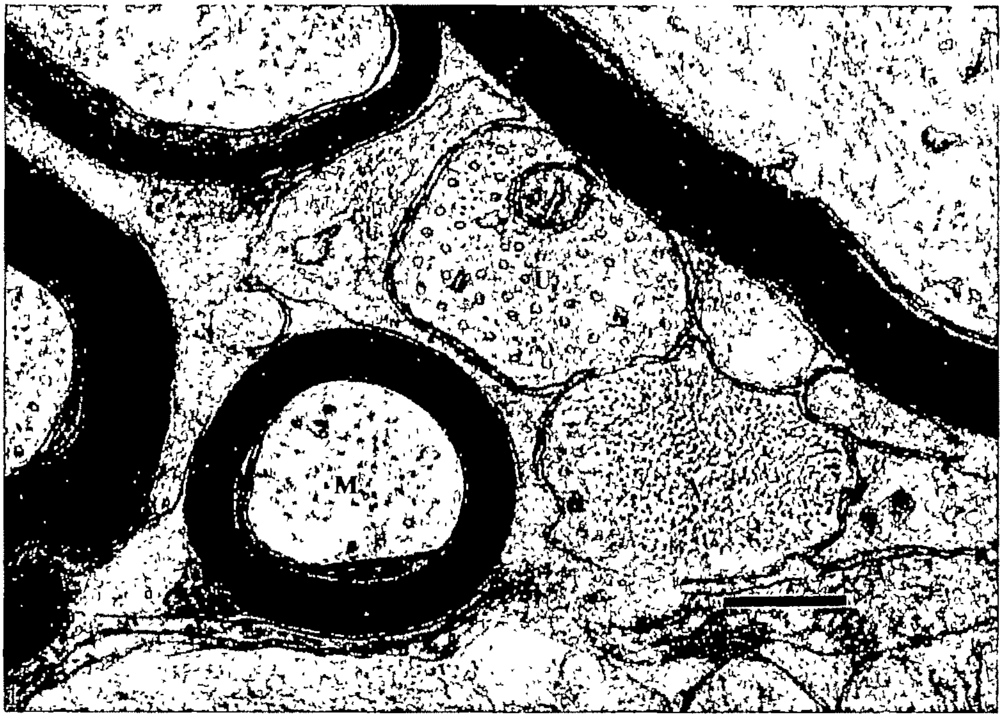

FIG. 1. Electron micrograph of a myelinated axon (M), an unmyelinated axon (U), and an astrocytic process (A). Note the microtubules and neurofilaments that characterize the axons and the concentration of microfilaments in the astrocytic process. Bar = 0.5 μm.

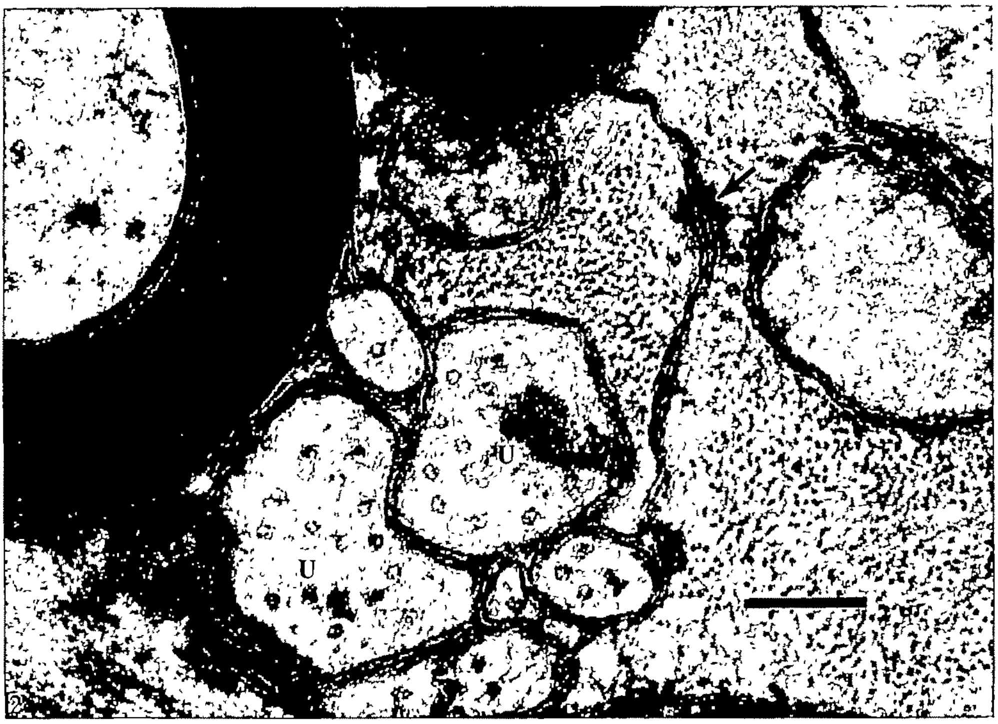

FIG 2. Group of unmyelinated axons (U), characterized primarily by microtubules, surrounded by astrocytic processes that contain numerous microfilaments. Note the typical desmosome (macula adhaerens) uniting the astrocytic processes (arrow). Bar = 0.5 μm

TABLE. NUMBERS OF AXONS IN THE POSTERIOR COLUMNS OF HUMAN SPINAL CORDS

Specimen	*Myelinated*	*Unmyelinated*	*Total*
H1	280,806	98,225 (26%)	379,031
H2	69,695	27,913 (29%)	97,608

Each figure is from both the right and left sides. Note that unmyelinated axons make up slightly more than 25% of the total. Exact levels of the cord could not be determined but H1 was at midsacral levels and H2 at lower sacral levels

swell with a resultant loss of the size and shape criteria for identification of the cell process. The numbers of myelinated and unmyelinated axons from the right and left posterior columns of the 2 cases that we were able to use are presented in the Table. Note that 26% and 29% of the axons in these 2 cases are unmyelinated.

Axons immunostained with CGRP antibody are common in the posterior columns (figs 3, 4). All labelled axons observed were unmyelinated, and they are

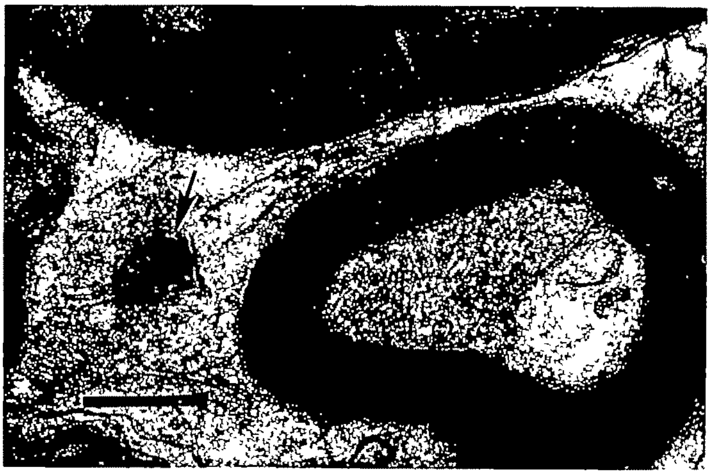

FIG. 3. Densely labelled unmyelinated axon (arrow) in the human dorsal funiculus. In this particular axon the reaction for CGRP is intense enough to obscure most of the cytoplasmic details. Bar = 0.5 μm.

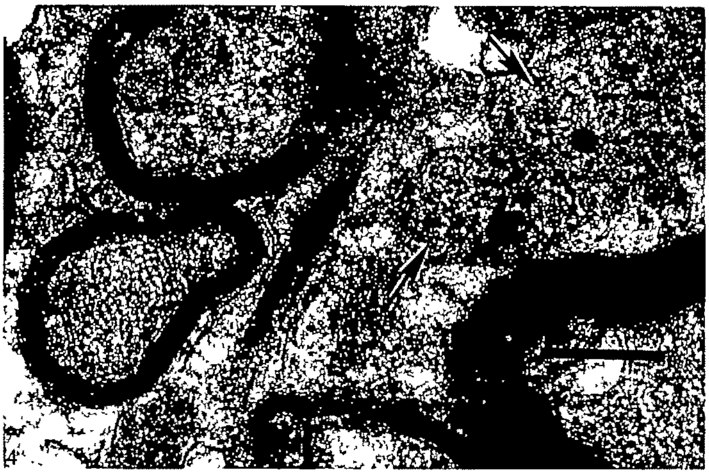

FIG 4. Two axons lightly labelled with CGRP reaction product (arrows). Bar = 0.5 μm.

found throughout the funiculus but especially in its lateral part. In heavily labelled axons, the labelled materials fills the axoplasm, thus obscuring cytological details (fig. 3), but in more lightly labelled axons, the reaction is associated with microtubules and small granules that are approximately 100 nm in diameter. These localizations are identical to those in rat and monkey (Carlton *et al.*, 1987; D. L. McNeill, R. E. Coggeshall and S. M. Carlton, unpublished observations). The labelled axons were numerous and in areas where staining was adequate made up at least 3% of the unmyelinated total.

DISCUSSION

Large myelinated fibres, the majority of which are ascending primary afferent axons, predominate in the mammalian dorsal funiculus. Unmyelinated fibres were also occasionally noted in classical papers (Nageotte, 1903, 1904; Tower, 1937; Tower *et al.*, 1941), but they were not emphasized and there has been little consideration of unmyelinated fibres as a significant component of dorsal funiculi in reviews or texts. This has clinical relevance in that the posterior columns may be stimulated for relief of pain on the basis that only large primary afferent fibres will be activated (Nashold and Friedman, 1972; Murphy and Giles, 1987). Thus it was a surprise to find unmyelinated fibres in the dorsal funiculi of the rat and cat (Langford and Coggeshall, 1981; Chung *et al.*, 1985, 1987). An obvious question was whether any of these unmyelinated fibres arose from dorsal root ganglion cells. We found that significant numbers were lost when the dorsal roots were cut and thus concluded that there is a significant contingent of unmyelinated primary afferent fibres in feline and rodent dorsal funiculi (Chung and Coggeshall, 1985; Chung *et al.*, 1987). The next question, which is the subject of the present paper, is whether unmyelinated sensory fibres can be found in the posterior columns in man.

To do this we must determine how many unmyelinated fibres are in the human posterior columns and provide evidence that at least some of them are primary afferents. The major problems that we faced assessing whether unmyelinated axons are a significant component of the human posterior columns are adequate fixation and adequate sampling. As is well known, perfusion of fixative is usually necessary for optimal neural fixation, but this option was not open to us. Accordingly we used immersion fixation as soon as feasible after death. The results were adequate in 2 of the 9 cases in which this was attempted. In these cases, the myelinated axons have their characteristic sheaths, and the smaller unmyelinated axons can be recognized by their size, 0.1 to 1.0 μm in diameter, their round or roughly oval shape on cross section, and their characteristic content of filaments and microtubules, along with occasional mitochondria and cisternae of agranular endoplasmic reticulum (Peters *et al.*, 1976). The only other neural processes in the column are dendrites which extend into the white matter, but they are usually considerably larger, have a radial orientation as compared with the longitudinal axons, and have presynaptic endings on them. Glial processes might also cause confusion, but these have a more irregular shape than axons, and they are characterized by large numbers of intermediate filaments, many more than in axons (Peters *et al.*, 1976). Thus although some uncertainty can occur, the large majority of processes are easily identifiable, and we conclude that our identifications are relatively accurate.

The second problem, that of sampling, was dealt with by counting all myelinated and unmyelinated axons in sample pictures taken throughout the funiculus, measuring the area of each picture and then multiplying the sums of the axon counts by the total area of the funiculus divided by the area sampled. This obviously leads to some sampling error, but this method has been used to determine axon numbers in large peripheral nerves (Dyck *et al.*, 1968), and it is extremely unlikely that the error is so great as to affect the conclusions. Accordingly we believe our sampling is adequate.

If the above reasoning is accepted, the conclusion would be that slightly more than 25% of posterior column axons in human sacral spinal cord are unmyelinated. This is the same general percentage as in rats and cats (Chung and Coggeshall, 1985; Chung *et al.*, 1985, 1987). On the basis of the results from the rat and cat, we hypothesized that many of the human axons are processes of dorsal root ganglion cells and thus are primary afferents. The most direct way of showing this would be to demonstrate the loss of unmyelinated axons following dorsal rhizotomy. Such material is not available to us. Accordingly we used a more indirect approach by showing that many fine fibres in the human posterior columns are immunostained by an antibody to CGRP. This is a compound that is found in many small dorsal root ganglion cells, in axons in the dorsal root and Lissauer's tract, and in axons and presynaptic terminals in laminae I and II of the dorsal horn (Rosenfeld *et al.*, 1983; Gibson *et al.*, 1984; Wiesenfeld-Hallin *et al.*, 1984; Lee *et al.*, 1985; Skofitsch and Jacobowitz, 1985*a*, *b*; Tschopp *et al.*, 1985; Gulbenkian *et al.*, 1986; Ruda *et al.*, 1986; Su *et al.*, 1986; Carlton *et al.*, 1987). The above localizations plus the observation that CGRP axons and terminals are almost completely lost from the dorsal horn and Lissauer's tract following dorsal rhizotomy (Gibson *et al.*, 1984; K. Chung and S. M. Carlton, unpublished observations) or capsaicin administration (Skofitsch and Jacobowitz, 1985*b*; Su *et al.*, 1986) led to its acceptance as a primary afferent marker. We find in this study that many unmyelinated axons in human posterior columns are labelled by the anti-CGRP. We therefore suggest that unmyelinated sensory fibres make up a significant part of human posterior columns. The functions of these fibres are not known, but they might be remembered when considering dorsal root entry zone lesions to relieve pain or asking why intense stimulation of the posterior columns can cause pain. The coexistence of substance P and CGRP in primary afferent neurons (Dalsgaard *et al.*, 1982; Wiesenfeld-Hallin *et al.*, 1984; Hökfelt *et al.*, 1986; Lundberg and Hökfelt, 1986; Gibbins *et al.*, 1987) might be of interest in this regard.

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THE CEREBRAL METABOLISM OF GLUCOSE AND OXYGEN MEASURED WITH POSITRON TOMOGRAPHY IN PATIENTS WITH MITOCHONDRIAL DISEASES

by R. S. J. FRACKOWIAK,[1,2] S. HEROLD,[1] R. K. H. PETTY[2] *and* J. A. MORGAN-HUGHES[2]

(From the [1]MRC Cyclotron Unit and Department of Medicine (Neurology), Hammersmith Hospital, and the [2]Institute of Neurology, London)

SUMMARY

Cerebral oxygen and glucose metabolism have been studied in 8 patients with biochemically defined mitochondrial myopathies and 7 normal control subjects using positron emission tomography. Four patients had myopathy alone and 4 had predominantly central nervous system (CNS) disease. Cerebral oxygen and glucose metabolism were measured concurrently in the two groups of patients with ^{15}O, ^{11}C and ^{18}F labelled tracers and positron emission tomography (PET). Patients with major CNS disease showed an uncoupling of glucose and oxygen metabolism when compared with patients without cerebral disease and normal subjects. The mean ratio between oxygen and glucose utilization was 3.8 moles of oxygen per mole of glucose in patients with CNS disease, compared with 5.6 for controls and 6.4 for patients with myopathy alone. The altered stoichiometry in CNS cases indicates aerobic glycolysis to lactate and/or other intermediate metabolites. Patients with major CNS disease showed a 50% reduction in cerebral oxygen utilization compared with cerebrally unaffected patients and normal subjects.

These findings indicate that in patients with a mitochondrial encephalopathy the defect identified in skeletal muscle is also expressed in the brain.

INTRODUCTION

Mitochondrial diseases are a heterogeneous group of inborn errors with diverse patterns of clinical expression and in which an increasingly large variety of underlying biochemical defects is being recognized (Morgan-Hughes, 1986*a*, *b*). One major subgroup is characterized by functional deficiencies of respiratory enzyme complexes (Morgan-Hughes *et al.*, 1987). Clinically, patients may be difficult to categorize because of the range of age of onset, the great variability in the degree of functional disability and the absence of any characteristic patterns of organ involvement (DiMauro *et al.*, 1985; Petty *et al.*, 1986). Defects of this type are typically associated with muscle weakness and fatiguability, a variable lactic

Correspondence to: Dr R. S J Frackowiak, MRC Cyclotron Unit, Hammersmith Hospital, 150 Ducane Road, London W12 0HS, UK.

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acidaemia and 'ragged-red' muscle fibres (Olson *et al.*, 1972). Some patients have central nervous system (CNS) disease which may present with dementia, cerebellar ataxia, seizures, involuntary movements, deafness or acute stroke-like episodes. Other patients with identical biochemical defects in muscle may show no CNS involvement. The variable phenotype encountered in these disorders could be explained by the presence of tissue specific isoforms of the respiratory enzyme complexes under the control of nuclear genes (A. H. V. Schapira *et al.*, unpublished results), or the presence of a heterogeneous population of normal and defective mitochondria in the ovum with random partitioning during embryogenesis (Egger and Wilson, 1983).

For obvious reasons these biochemical defects have been identified in easily accessible tissues suitable for biopsy and the in vitro isolation of functionally intact mitochondrial samples. Positron emission tomography (PET) provides a quantitative tracer method to measure tissue energy metabolism in inaccessible tissues in vivo (Phelps *et al.*, 1986). Techniques have been well described, validated and applied to man for measurement of the regional metabolism of oxygen (Frackowiak *et al.*, 1980; Baron *et al.*, 1981; Lammertsma *et al.*, 1981, 1982, 1983; Rhodes *et al.*, 1981; Lammertsma and Jones, 1983*a*) and glucose in the brain (Phelps *et al.*, 1979*b*; Reivich *et al.*, 1979). The measurement of glucose metabolism depends on the steady-state measurement of the flux of glucose through the first (hexokinase) step of the glycolytic pathway. On the other hand, the technique for making measurements of oxygen consumption depends on the generation of metabolic water in respiring tissue by the acceptance of reducing equivalents transferred down the respiratory chain (Jones *et al.*, 1976). The combination of these two measurements provides a method for the analysis of cellular bioenergetics in vivo (Baron *et al.*, 1984). The normal molar stoichiometry between oxygen and glucose utilization in the human brain is 5.6 (Lassen, 1959). The remaining 0.4 moles are accounted for by other routes of glucose metabolism, for example to lactate or other intermediate metabolites. Marked deviations from this relationship would be expected when oxidative metabolism is impaired. In such cases less oxygen than normal will be consumed per mole of glucose metabolized.

The aim of the study was to determine whether there were differences in oxidative metabolism in the brains of patients with identified biochemical defects in muscle mitochondria who differed in their clinical expressions.

MATERIAL AND METHODS

Clinical details

Seven normal volunteers, 5 males and 2 females aged between 23 and 53 yrs (34 ± 10.5, mean ± SD) were studied. They had no history of previous neurological or psychiatric illness, or general medical disease. All were either graduates in gainful employment or full-time students.

Seven patients with mitochondrial respiratory chain deficiencies and 1 patient with H^+-ATPase deficiency were studied: 4 had evidence of major CNS disease and 4 had myopathy alone. The 4 with CNS disease were aged 23 to 64 yrs (48 ± 18, mean ± SD); there were 3 males and 1 female. The 4 with no

TABLE 1 BIOCHEMICAL DEFECTS IDENTIFIED IN FRESHLY ISOLATED MUSCLE MITOCHONDRIA FROM 8 PATIENTS

Case (No.)	*Locus of biochemical defect*
Without CNS disease	
1 (927)	NADH CoQ reductase (complex I)
2 (878)	NADH CoQ reductase (complex I) + carnitine deficiency
3 (939)	NADH CoQ reductase (complex I) + carnitine deficiency
4 (933)	NADH CoQ reductase (complex I) + cytochrome oxidase (complex IV)
With CNS disease	
5 (748)	NADH CoQ reductase (complex I)
6 (911)	Mitochondrial H^+-ATPase + carnitine deficiency (complex V)
7 (1206)	NADH CoQ reductase (complex I)
8 (846)	NADH CoQ reductase (complex I)

signs of CNS disease were aged 21 to 56 yrs (38 ± 17, mean ± SD); there were 3 females and 1 male. The locus of the biochemical defect in each case is shown in Table 1. Biochemical studies were carried out on freshly isolated skeletal muscle mitochondria according to previously described methods (Morgan-Hughes *et al.*, 1982).

Case histories

No CNS disease

Case 1 (*No. 927*). A 21-yr-old woman with weakness and exercise intolerance since early childhood. Her birth and early development were normal. She walked at 1 yr but tired easily and was less active than her peers. She was unable to run or play games at school and at the age of 11 yrs she developed unexplained congestive heart failure which resolved spontaneously after several months. The weakness and fatigue became worse in her late teens and she developed exercise-induced myalgia in her calves. Examination revealed generalized weakness with fatiguability of the sternomastoid, limb and trunk muscles with normal reflexes and flexor plantar responses. There was no clinical or laboratory evidence of CNS, retinal or cardiac involvement. The serum CK was 199 IU/l (normal < 80 IU/l), an ECG was normal and an echocardiogram revealed a bicuspid aortic valve. A CT brain scan was normal. EMG studies showed myopathic potentials with normal motor and sensory conduction. A standard 10 min exercise test on a bicycle ergometer showed a rise in venous blood lactate from 1.3 to 5.7 mmol/l after 10 min which was still elevated at 3.8 mmol/l after 30 min. A muscle biopsy showed 34% of 'ragged-red' fibres containing elongated mitochondria with transverse cristae. Her sister died aged 23 yrs of cardiorespiratory failure and severe lactic acidosis (Morgan-Hughes *et al.*, 1985, Case 1; Wiles *et al.*, 1986).

Case 2 (*No. 878*). A 56-yr-old woman with a 40 yr history of muscle fatiguability and weakness, and myalgia. For several years she had noticed difficulty in climbing stairs and getting out of a low chair. On examination, she had mild diffuse weakness of the limbs, more marked proximally. The reflexes and plantar responses were normal. The serum CK, ECG and CT brain scan were normal. A muscle biopsy showed occasional 'ragged-red' fibres and increased neutral lipid droplets in type 1 and 2a fibres. Total muscle carnitine measured 1.14 μmol/g wet weight (control mean ± SD = 2.0 ± 0.7, n = 28). Her daughter was similarly affected (Clark *et al.*, 1984).

Case 3 (*No. 939*). A 49-yr-old man with a 2 yr history of progressive weakness, severe exercise intolerance and proximal myalgia. When first seen he could only walk about 100 yards on the flat before having to rest and could climb only one flight of stairs. On examination he had weakness with fatiguability affecting the sternomastoid, limb and trunk muscles and was unable to rise from a squatting position without help. The reflexes were sluggish but the plantar responses were flexor. An

ECG and serum CK were normal. During a standard exercise test the venous blood lactate rose from 0.3 mmol/l to 3.0 mmol/l after 15 min. The EMG was myopathic; nerve conduction velocities were normal. Muscle biopsy showed the changes of a lipid storage myopathy with numerous 'ragged-red' fibres. Total muscle carnitine was low at 0.22 μmol/g wet weight (controls 2.0 ± 0.7) but plasma carnitine was normal (Morgan-Hughes *et al.*, 1985).

Case 4 (*No. 933*). A 27-yr-old female with weakness and exercise fatiguability since childhood and ptosis since her late teens. She walked at 18 months and fell frequently during childhood. She was unable to run or play games at school and had intermittent diplopia when tired. Menarche was delayed until she was 20 yrs of age. On examination she had bilateral ptosis, marked limitation of extraocular movements, with facial, sternomastoid and diffuse limb and trunk weakness. The reflexes and plantar responses were normal and sensation was intact. The serum CK, CSF, ECG and a CT brain scan were normal EMG studies showed myopathic potentials; nerve conduction velocities were normal. Venous lactate levels rose from 0.9 to 4.5 mmol/l during a standard exercise test. Muscle biopsy showed 20% 'ragged-red' fibres.

With CNS disease

Case 5 (*No. 748*). A 46-yr-old man with a 6 yr history of progressive deafness, forgetfulness, personality change and ataxia of gait. On examination he was euphoric and demented (verbal IQ 67; performance IQ 80), with bilateral sensorineural deafness, scanning cerebellar dysarthria, a broad-based ataxic gait and choreic limb movements. Muscle bulk and power were normal. Sensation and reflexes were normal but the plantar responses were extensor. EMG studies showed evidence of denervation in the distal limb muscles and absent sensory nerve action potentials but normal motor conduction A CT brain scan showed cerebral and cerebellar atrophy with areas of low attenuation in white matter The serum CK, CSF, ECG and EEG were normal. Venous blood lactate levels rose to 1.9 mmol/l after 10 min exercise. A muscle biopsy showed minor neurogenic changes with 30% 'ragged-red' fibres (Morgan-Hughes *et al.*, 1985, Case 3).

Case 6 (*No. 911*). A 23-yr-old male was normal until aged 5 yrs when he developed intermittent headaches, recurrent episodes of unexplained vomiting, easy fatiguability, exertional dyspnoea and intellectual deterioration. These symptoms progressed until the age of 9 when he had a generalized seizure. Investigations at this time showed calcification of the basal ganglia on CT, raised CSF protein, sensorineural hearing loss and short stature. Muscle biopsy showed a lipid storage myopathy with 'ragged-red' fibres and a muscle carnitine concentration of 2.6 μmol/g dry weight (Smyth *et al.*, 1975). On treatment with oral carnitine the vomiting stopped and there was gradual improvement in muscle strength, but he went on to develop progressive intellectual deterioration, cerebellar ataxia and a peripheral sensory neuropathy. On examination he was demented (verbal IQ 71; performance IQ 40) and dysarthric, with a broad-based ataxic gait. He had a pigmentary retinopathy of the 'salt-and-pepper' type, mild facial weakness and diffuse weakness with fatiguability in the limbs with hyporeflexia and extensor plantar responses Vibration and position sense was impaired in the toes and fingers. The serum CK and ECG were normal. EMG studies showed myopathic potentials with an axonal sensorimotor neuropathy. A CT brain scan showed generalized atrophy with calcification of the basal ganglia. An EEG showed paroxysmal slow wave activity. Repeat muscle biopsy at 17 yrs of age showed disappearance of lipid storage and 20% 'ragged-red' fibres. Total muscle carnitine concentration was also normal (Clark *et al*, 1984, Case 1).

Case 7 (*No. 1206*). A 58-yr-old man presented with an 8 yr history of progressive deafness and ataxia of gait. For 3 yrs he had had difficulty in climbing stairs and for a year had noticed poor memory. Since his twenties he had frequently complained of lethargy and fatigue and when aged 31 had developed insulin-dependent diabetes. On examination he was mildly demented (verbal IQ 85; performance IQ 84) with an ataxic gait, cerebellar dysarthria and severe sensorineural deafness. There was mild diffuse weakness with fatiguability in the limbs with reduced muscle bulk, absent tendon reflexes and flexor plantar responses. Pain and light touch were diminished in a glove and stocking distribution. The serum CK was 167 IU/l (normal < 80 IU/l), random venous blood lactate levels were 3.0 and

2.6 mmol/l (normal up to 1.8 mmol/l) and the CSF protein was 0.6 g/l. An EEG showed left ventricular hypertrophy and inverted left ventricular T waves. A CT brain scan showed cerebral and cerebellar atrophy. EMG studies showed a motor and sensory axonal neuropathy. Muscle biopsy showed mild denervation and about 40% 'ragged-red' fibres.

Case 8 (*No 846*). A 64-yr-old female with a 3 yr history of progressive dysarthria, ataxia of gait and impaired memory. On examination, she was small (140 cm) and mildly demented (verbal IQ 91; performance IQ 72). There was marked limb and gait ataxia and mild weakness with fatiguability of proximal limb muscles. The tendon reflexes were brisk and the plantar responses extensor, but sensation was normal. The serum CK was normal. EMG studies showed myopathic potentials. Nerve conduction velocities were normal A CT brain scan showed cerebral, brainstem and cerebellar atrophy with areas of low attenuation in white matter. Muscle biopsy showed mild myopathic changes and 20% 'ragged-red' fibres.

Positron tomography

Patients and normal volunteers were scanned with oxygen-15 labelled tracers ($^{15}O_2$ and $C^{15}O_2$) and with fluorine-18 labelled 2-deoxy-D-glucose (^{18}FDG) Selected studies with carbon-11 labelled carbon monoxide (^{11}CO) were also performed (Phelps *et al.*, 1979*a*).

All measurements were made with the ECAT II tomograph (CTI Inc., Knoxville, Tennessee) with a transaxial resolution at full width half maximum (FWHM) of 1.6 cm in x and y axes and 1.7 cm in the z axis. Patients and subjects were scanned recumbent with minor head restraint and constant supervision of head immobility. The eyes were closed and ears unplugged in conditions of minimum ambient noise and tactile stimulation. A 21 gauge, 3 cm Teflon catheter was placed in a radial artery before positioning the subject in the tomograph. All subsequent blood samples were taken without disturbing the subject. An intravenous catheter was placed in the contralateral arm for the administration of ^{18}FDG. Gaseous tracers were administered through a loose fitting plastic face mask. Scans were performed through one transaxial plane at the level of the insula and basal ganglia (OM + 4–OM + 5 cm) only. Positioning was determined from a short emission scan during the initial phase of inhalation of ^{15}O-labelled gas.

Oxygen consumption

The steady state inhalation method was used for the measurement of oxygen metabolism (CMR_{O_2}) and blood flow (CBF) (Frackowiak *et al.*, 1980; Lammertsma and Jones, 1983). Trace quantities of $C^{15}O_2$ were continuously inhaled by the patient. Within 10 min a dynamic steady-state was reached during which radioactivity recorded from the head remained constant with time. The plane of interest was scanned for 5 min allowing the accumulation of 1.0–1.5 × 10^6 counts. During scanning, 3 arterial samples were drawn for measurement of plasma and whole blood activity. The inhalation was stopped and after waiting a further 10 min for the activity to decay away ($t_{\frac{1}{2}}\,^{15}O = 2.1$ min), an inhalation of $^{15}O_2$ was commenced and a second scan recorded at a similar dynamic equilibrium. The $^{15}O_2$ scan was followed by a transmission scan, using a ^{68}Ge/^{68}Ga external ring source, for the purposes of correcting all the emission scans accurately for attenuation.

Glucose consumption

Cerebral glucose consumption (CMR_{Glu}) was measured with a kinetic modification of the Sokoloff analogue tracer technique (Sokoloff *et al.*, 1977) using ^{18}FDG (Lammertsma *et al.*, 1987). Following decay of the ^{15}O activity, between 3 and 7 mCi of ^{18}FDG in 10 ml of saline were given intravenously by Harvard pump over 2 min. Simultaneously, arterial blood sampling for measurement of radioactivity was started and performed every 20 s for 3 min, 30 s for a further 2 min, every 1 min until 10 min, every 3 min until 20 min and then at 5 min intervals until the end of scanning at 50–60 min. Scanning was commenced at the beginning of ^{18}FDG infusion with five consecutive 1 min scans followed by six 5 min scans and ending with a 5–10 min scan starting 45 min after injection of tracer. The last scan generated 0.3–0.8 × 10^6 counts in the plane of interest. Four plasma glucose measurements were also made.

Blood volume and correction of CMR_{O_2}

Finally, in 2 normal subjects and 6 patients a 2 min inhalation of ^{11}CO was followed by a 5 min equilibration period and a final scan to determine the distribution of the blood volume tracer (Phelps *et al.*, 1979*a*). As the half life of ^{18}F is 110 min, residual background activity from the ^{18}FDG procedure contaminated the ^{11}CO signal. This contaminating activity was therefore removed from the ^{11}CO data by decay correcting the final ^{18}FDG scan to the time of the ^{11}CO scan and subtracting. This, and all the corrections involving tracer distributions, was made possible because a number of techniques were used to ensure that the subject's head remained immobile throughout the study and hence that the activity distributions were spatially congruent. Three blood samples were obtained to calculate the cerebral blood volume (CBV) from the corrected ^{11}CO scan. A value of 0.85 was taken from the literature for the peripheral cerebral to venous haematocrit ratio necessary for calculation of CBV (Larsen and Lassen, 1964) The CBV is required to correct the $^{15}O_2$ data for intravascular activity so that fractional oxygen extraction and hence CMR_{O_2} can be accurately calculated (Lammertsma and Jones, 1983). It proved impossible to perform ^{11}CO scans on all subjects and patients. In those in whom it was not done, a value for CBV was derived from the corresponding value for CBF. It is known from previous studies that there is normally a close correlation between CBF and CBV (Grubb *et al.*, 1977). In each of the three groups the CBF/CBV ratio was calculated from the available, measured, complete data sets. A CBV was then calculated for those in whom it had not been measured, from the measured CBF and the mean group CBF/CBV ratio. The correction for intravascular $^{15}O_2$ is important as its lack will cause an overestimation in OER and hence CMR_{O_2} by approximately 20%. However, correction with an estimated CBV, even if in error by as much as 15%, will result in at most an error of 1–2% in CMR_{O_2} (Lammertsma and Jones, 1983; Lammertsma *et al.*, 1983). This approximation is therefore justified and was applied to 1 patient in each of the 2 patient groups and to 5 of the 7 normal subjects.

Blood volume and calculation of CMR_{Glu}

The CBV data are also necessary for subtraction of vascular activity from the ^{18}FDG scans, particularly early on when the tissue signal is contaminated significantly by intravascular tracer. This subtraction allows the accurate construction of an extravascular tissue time-activity curve from the sequentially collected scan data for any region of interest in the scanned plane. The tissue uptake of tracer in response to the vascular tracer input function, which is measured by blood sampling, can therefore be described. A kinetic, least squares, computerized curve-fitting procedure permitted the unique solution of the data in terms of 4 kinetic rate constants. These describe the transport of the tracer from blood into tissue and in the reverse direction (k_1^* and k_2^*), phosphorylation of free tracer in the tissue by hexokinase and any hydrolysis of the accumulated product that may occur (k_3^* and k_4^*). The curve analysis was rendered more accurate by fitting for the product $[k_1^* k_3^*/(k_2^* + k_3^*)]$ directly. This expression when multiplied by the plasma glucose concentration and divided by a 'lumped constant' (LC), which is a factor that relates the behaviour of the analogue tracer ^{18}FDG to the natural substrate glucose, gives the CMR_{Glu},

i.e. $$CMR_{Glu} = \{[\text{glucose}]_p/LC\} \times \{k_1^* k_3^*/(k_2^* + k_3^*)\}$$

where $[\text{glucose}]_p$ is the plasma glucose concentration. The LC used in these studies was derived empirically from knowledge of the molar relationship between oxygen and glucose consumption in the brains of normal young men, which has been shown to be 5.6 (Lassen, 1959). LC was therefore calculated using the CMR_{O_2} data from the normal subjects in this study. The value of this empirical 'lumped constant' (LC′ = 0.75) is higher than that conventionally used (0.42, Phelps *et al.*, 1979*b*) or that measured using arteriovenous difference techniques in 3 volunteers by Reivich *et al.*, 1979 (0.54). The reasons and justification for this approach will be found in the Discussion.

The total time taken to collect a set of measurements with the PET scanner was under 2 h without a CBV scan and under 2.25 h if one was included. The scans were analysed using the cortical plot technique previously described (Wise *et al.*, 1983). This centres on 2 hemisphere middle cerebral artery regions of interest of 1.5 cm width. They encompass the area of maximal peripheral activity in the scan corresponding to the cortical ribbon, but which also include some underlying white matter. The results from the 2 hemisphere plots were averaged for each subject as no significant right/left asymmetries were discovered.

The protocols were approved by the research ethics committee of the Hammersmith and National Hospitals and by the Administration of Radioactive Substances Advisory Committee of the DHSS.

RESULTS

Blood biochemistry is tabulated for normal subjects and patients in Table 2.

Normal subjects

The results in normal subjects are presented in Table 3. The mean CMR_{O_2} for the group of 7 normal subjects was found to be 153 ± 14.5 μmol/dl/min (3.4 ± 0.3 ml/dl/min), the CMR_{Glu} was 27.4 ± 2.4 μmol/dl/min (4.9 ± 0.4 mg/dl/min) and the molar ratio (MR) between the two was 5.6 ± 0.6. As described above, the LC′ used in calculations of CMR_{Glu} was derived empirically from an assumed normal MR of 5.6, the measured CMR_{O_2}, the kinetic rate constants obtained from ^{18}FDG scanning and the plasma glucose concentrations in the 2 subjects in whom CBV was also measured. This value when applied to the whole group of 7 normal subjects resulted in a mean MR of 5.6, indicating the consistency of the approach. The group CBF was 43.9 ± 7.4 ml/dl/min which is slightly lower than that found in smaller and more homogeneous grey-matter regions in normal volunteers. This indicates that the

TABLE 2 BLOOD BIOCHEMISTRY IN NORMAL SUBJECTS AND PATIENTS

Case no.	*pO_2 (kPa)*	*pCO_2 (kPa)*	*Hb (g/dl)*	*Plasma glucose (mmol/l)*
Normal subjects				
953	11.96	6.30	15.0	6.9
958	11.85	5.65	15.0	5.0
964	13.20	5 30	14.4	5.2
981	11.70	5.50	13.4	5.0
998	13.60	5.40	14.0	4.6
1006	12.50	5.65	13.3	5.5
1050	12.75	5.20	12.8	5.3
Without CNS disease				
1 (927)	11.65	5.20	13.5	4.9
2 (878)	10.55	5.10	14.2	6.7
3 (939)	10.30	5.35	13.7	5.7
4 (933)	12.90	5.20	12.4	5.0
With CNS disease				
5 (748)	14.05	5.25	12.9	5.3
6 (911)	13.30	5.20	13.0	6.2
7 (1206)	10.35	6.15	12.4	12.9
8 (846)	9.75	5.95	9.9	5.5

TABLE 3 VALUES OF PHYSIOLOGICAL VARIABLES IN 7 NORMAL SUBJECTS

Subject no.	CMR_{O_2} (*μmol/dl/min*)	CMR_{Glu} (*μmol/dl/min*)	*MR*	*CBF* (*ml/dl/min*)	*OER*	*GER*
953*	148	26.2	5.6	39.3	0.42	0.10
958*	142	25.3	5.6	40.8	0.39	0.12
964	150	23.8	6.3	56.6	0.31	0.08
981	147	29.3	5.0	40.9	0.44	0.14
998	185	29.0	6.4	51.6	0.42	0.12
1006	147	27.5	5.3	35.8	0.51	0.14
1050	149	30.4	4.9	42.1	0.45	0.14
Mean	153	27.4	5.6	43.9	0.42	0.12
SEM	±14.5	±2.4	±0.6	±7.4	±0.06	±0.02

* CBV measured and LC′ derived from complete data set by assuming the normal MR to be 5.6 (*see* text). Using this LC′ to calculate CMR_{Glu} in the remaining subjects results in a group mean MR which is also 5.6

TABLE 4 VALUES OF PHYSIOLOGICAL VARIABLES IN PATIENTS WITH MITOCHONDRIAL MYOPATHY WITHOUT EVIDENCE OF CNS DISEASE

Case no.	CMR_{O_2} (*μmol/dl/min*)	CMR_{Glu} (*μmol/dl/min*)	*MR*	*CBF* (*ml/dl/min*)	*OER*	*GER*
1	185	23.3	7.9	44.0	0.51	0.10
2	156	25.3	6.2	36.6	0.49	0.10
3*	120	22.0	5.4	37.4	0.39	0.10
4	145	23.4	6.2	40.1	0.49	0.12
Mean	152	23.5	6.4	39.5	0.47	0.11
SEM	±27	±1.4	±1.1	±3.3	±0.05	±0.01

* CBV was estimated rather than directly measured (*see* text).

method of cortical strip analysis results in some contamination of cortical grey matter by underlying white matter, an inevitable consequence of the relatively coarse resolution of the ECAT II PET camera. The fractional oxygen extraction (OER) was 0.42±0.06 and the fractional glucose extraction (GER) 0.12±0.02.

Patients without cerebral symptoms

The results in patients with respiratory chain defects without cerebral symptoms are presented in Table 4. The mean CMR_{O_2} and CMR_{Glu} were 152±27 μmol/dl/min and 23.5±1.4 μmol/dl/min (3.4±0.6 ml/dl/min and 4.2±0.3 mg/dl/min) respectively, giving an MR of 6.4±1.1. The mean CBF was 39.5±3.3 ml/dl/min, the OER 0.47±0.05 and the GER 0.11±0.01.

Patients with cerebral symptoms

The results in patients with mitochondrial myopathy and CNS disease are given in Table 5. Mean CMR_{O_2} and CMR_{Glu} were 77.1±15.2 μmol/ml/min and 20.3±1.9 μmol/ml/min (1.7±0.3 ml/dl/min and 3.7±0.3 mg/dl/min), resulting in a very

TABLE 5. VALUES OF PHYSIOLOGICAL VARIABLES IN PATIENTS WITH MITOCHONDRIAL CYTOPATHY AND CEREBRAL DISEASE

Case no.	*CMR_{O_2} (µmol/ml/min)*	*CMR_{Glu} (µmol/ml/min)*	*MR*	*CBF (ml/dl/min)*	*OER*	*GER*
5	64.5	21.9	2.9	29.3	0.26	0.14
6	69.9	17.7	3.9	33.1	0.27	0.09
7*	75.2	21.4	3.5	21.8	0.45	0.08
8	98.9	20.2	4.9	38.3	0.44	0.10
Mean	77.1	20.3	3.8	30.6	0.36	0.10
SEM	±15.2	±1.9	±0.8	±6.9	±0.10	±0.03

* CBV was estimated rather than directly measured (*see* text).

TABLE 6 VALUES OF KINETIC RATE CONSTANTS OBTAINED FROM THE THREE GROUPS OF SUBJECTS ($\times 10^{-2}$)*

	Normal subjects	*Mitochondrial myopathy without CNS disease*	*Mitochondrial myopathy with CNS disease*
k_1^*	9.17± 1 37	8 16±1.62	6.47±1.19
k_2^*	22.2 ±11.46	16.49±2.85	18.28±3.68
k_3^*	15.9 ± 7.24	10 83±1.71	10.23±4.77
k_4^*	1.21± 0.41	1.28±0.54	1.71±0.36
$\frac{k_1^* k_3^*}{k_2^* + k_3^*}$	3.89± 0.65	3.20±0.39	2.31±0.81

* Constants obtained by averaging individual values, each obtained by curve fitting tissue to arterial blood counts obtained from and during PET scanning The tissue counts are corrected for intravascular activity. The rate constants are in units·min^{-1}

significantly decreased MR of 3.8±0.8 ($P < 0.005$). The mean CBF was 30.6±6.9 ml/dl/min, the OER 0.36±0.1 and the GER 0.10±0.03. The large spread in the OER value was due to a low value in 2 of the patients and a normal value in the 2 others.

FDG kinetic constants

The kinetic constants for the 3 groups are presented in Table 6. The ratio $[k_1^* k_3^*/(k_2^* + k_3^*)]$ is used to calculate CMR_{Glu}. The curve-fitting procedure used to calculate this function permits direct estimation of the standard error (SE) for each fit, which gives an estimate of the SE of the calculated value of CMR_{Glu}. The SE was 4.4±2.2% in the normal group, 6.7±2.8% in those without cerebral symptoms and 8.4±2.8% in those with cerebral symptoms giving a mean SE of 6.2±2.9%. The rate of apparent hydrolysis (k_4^*) was very similar in all 3 groups and approximately 11% that of phosphorylation (k_3^*). Curve fitting with only 3 constants, ignoring k_4^*, gave less good fits for the rate constants and the function $[k_1^* k_3^*/(k_2^* + k_3^*]$.

TABLE 7 ANALYSIS OF VARIANCE (ONE-WAY ANOVA) BETWEEN VARIABLES AND GROUPS

Variable	*F ratio between groups**	*Probability > F*
CBF (ml/dl/min)	5.290	0.023
CMR_{O_2} (μmol/dl/min)	23.99	0.00
CMR_{Glu} (μmol/dl/min)	15.781	0.00
OER	2.511	0.123 (n.s.)
MR	11.669	0.002

* Normal subjects, cerebrally affected and cerebrally normal patients There is a significant difference between the groups for all variables except OER

Statistical analysis

An analysis of variance (one way ANOVA) was performed to assess the significance of differences between groups (Table 7). The significance of differences in MR between patients and normals was then tested with a two-tailed Student's t test.

DISCUSSION

These results provide strong direct evidence that in patients with mitochondrial encephalopathy the functional defect identified in freshly isolated skeletal muscle is also expressed in cerebral tissue. The marked decrease of over a third in the molar ratio indicates that in these patients approximately 4 molecules of oxygen, rather than the expected 6, are consumed per molecule of glucose. This implies the aerobic glycolysis of up to a third of the glucose metabolized in the brain, presumably to lactate and other intermediate metabolites (e.g., pentose phosphate shunt intermediates and 2-carbon metabolic products of the Krebs cycle). This deranged metabolism of glucose is occurring in the context of normal tissue oxygenation as demonstrated by the normal, and sometimes subnormal, fractional extraction of oxygen (OER). If oxidative phosphorylation is impaired due to a primary defect of mitochondrial function, excess ADP and/or $NADH^+$ will stimulate glycolysis via phosphofructokinase. The results support this hypothesis. The rate of oxygen metabolism was profoundly depressed in the patients with CNS disease and correlated with the rank order of dementia (estimated with the WAIS) in the 4 patients. The depression of CMR_{O_2} of 50% contrasts with the much lower reduction in the CMR_{Glu} of only 26% and with the normal CMR_{O_2} in the patients without CNS disease.

A second observation in the group with CNS disease is the presence of a low OER in the 2 patients with the severest cerebral disease. This implies a disturbance in the normal coupling between perfusion and energy metabolism in these patients. The mechanism for this is unclear at present. A possibility is that local cerebral lactate production may be high with perhaps an associated tendency to cerebral lactic acidosis. However, Berkovic *et al.* (1987) have recently described, in an abstract, studies in 3 patients with myoclonic epilepsy and 'ragged-red' fibres and noted no change in cerebral pH (measured with PET) and a normal intracellular pH measured in 1 patient with MRI.

The molar ratio in patients with respiratory chain defects but without CNS disease was 14% higher than in normal subjects, although the difference was not statistically significant. It is known that glucose is the preferred and normally the virtually exclusive substrate of the brain for energy production. However, in ketotic states ketone bodies can function as alternative substrates because uptake is passive and concentration dependent. Patients with respiratory chain defects frequently develop ketonaemia (*see* clinical descriptions above). It is therefore possible that in some patients without CNS disease and with apparently normal cerebral energy metabolism, the slight excess of oxygen molecules consumed for the amount of glucose metabolized (i.e., > 6) is explained by oxidation of ketone bodies.

There is little or no information about the stoichiometry between glucose and oxygen metabolism measured in the same individuals with other dementing conditions. Alzheimer's disease is known to be associated with a reduction of 20–40% in CMR_{O_2} that is correlated with the severity of the dementia (Frackowiak *et al.*, 1981). Similar findings have been reported from a number of laboratories for glucose metabolism (for review, *see* Frackowiak, 1987). The literature does not therefore support the suggestion that dementia per se is associated with a differential impairment of oxygen and glucose metabolism. The pattern observed in this study is probably specific to respiratory chain defects. One study (J. Hatazawa, personal communication), measuring CMR_{O_2} and CMR_{Glu} in the same patients suffering from Alzheimer's disease, showed apparently little change in the molar ratio with, if anything, a tendency towards an increase.

No focal abnormalities were observed in the cortical grey matter of subjects which could be related to different focal signs or symptoms. However, the data were collected on a tomograph with single-slice scanning capacity and had to be restricted for practical reasons to measurements from 1 plane only on this account. The possibility that focal changes might be observed needs to await further studies using multislice machines. A number of patients develop CNS symptoms and signs a considerable time after muscle disease first declares itself. The question arises whether such patients show disturbances of cerebral energy metabolism before the onset of overt CNS disease. The answer to this question also awaits further studies.

The results of the study are clear cut, but certain methodological features require discussion. The use of an empirically derived 'lumped constant' (LC′) might be criticized. However, the LC used conventionally (0.42) was likewise defined empirically by reference to a normal CMR_{Glu} for human brain measured by arteriovenous sampling techniques (Phelps *et al.*, 1979*b*). More recently, an LC of 0.54 has been measured in 3 normal subjects by a direct arteriovenous sampling method (Reivich *et al.*, 1985). An LC′ of 0.75 was used in this study. This is a combination of the true LC (which cannot be measured in each study) and an empirical correction factor and allows for a meaningful comparison of the CMR_{Glu}

and CMR_{O_2} results. The empirical nature of LC′ is justified on the grounds that it uses direct whole brain measurements of CMR_{Glu} obtained with arteriovenous difference methods for calibration, a procedure analogous to that of Phelps *et al.* (1979*b*). The major reason why an LC′ higher than the true LC is necessary is that the mean cortical CMR_{O_2} measured by the steady-state oxygen inhalation technique may be underestimated by as much as 20% because of the admixture of grey and white matter in the region of interest (Lammertsma *et al.*, 1981). As a result, the LC′ will be 20% higher if it is defined in terms of CMR_{O_2} values measured by the steady-state method. A second consideration is the effect that the measurement of CBV to correct for intravascular activity during ^{18}FDG scanning has on the LC. The measurement of CBV depends on knowledge of the peripheral venous to cerebral vessel haematocrit ratio. This has been conventionally taken as 0.85, the average of a number of studies in various animal species (Larsen and Lassen, 1964). Recently we have measured cerebral haematocrit with PET and found that the value of the haematocrit ratio in man is much closer to 0.7 (Lammertsma *et al.*, 1984). The use of this ratio, instead of the conventional 0.85 for the calculation of CBV, results in an overestimation of CMR_{Glu} by up to a maximum of 10%. This serves to increase LC′ in relation to LC by a further 10%. Thus the empirically derived LC′ of 0.75 we have used compares favourably with the measured 0.54 adjusted upwards by 30% (0.70) to compensate for the above factors. Finally, there may be a small effect on the LC by virtue of the fact that chemical preparations of ^{18}FDG have until very recently been contaminated by up to 20% ^{18}F deoxymannose. In conclusion, as the aim of this study was to investigate the relationship between oxygen and glucose metabolism, the use of a pragmatic LC′, defined empirically as described and applied to all the data seems adequately justified. It is notable that others who have attempted stoichiometric measurements in the past have consistently underestimated the molar ratio due to a combination of factors dependent on the precise methods of measurement (Baron *et al.*, 1984).

We have measured CMR_{Glu} kinetically. The rate constants are somewhat different to standard published values in normals (*see* Mazziotta and Phelps, 1986). There are several reasons for this. (1) The blood data in this study were obtained by arterial sampling. Others have frequently obtained these data by sampling from a heated, 'arterialized' hand vein. The former technique is more accurate (Budinger *et al.*, 1985), although many of the published rate constants have been measured using venous sampling (Phelps *et al.*, 1979*b*; Reivich *et al.*, 1979). (2) The correction for attenuation in this study has been measured rather than geometrically manipulated, which is also more accurate; this should not, however, affect the rate constant values significantly. (3) A correction for intravascular tracer has been applied by measurement of CBV, which has not been uniformly practised in the past. This is particularly important in kinetic analysis of the earliest phases after tracer injection when the intravascular activity dominates tissue activity. The CBV correction by measurement therefore makes the estimation of k_1^* more accurate. (4) The introduction of tracer by pulse injection may result in inadequate sampling of blood radioactivity during the initial short-lived peak of blood activity. We have therefore infused the tracer slowly over 2 min, smoothing out the initial phase of the input function, and allowing its more accurate definition by 20 s blood sampling over the first 3 min. (5) The study time of up to 60 min makes the accurate estimation of k_4^* difficult as hydrolysis is a slow and largely insignificant process at this time (Sokoloff *et al.*, 1977). This results in a tendency to overestimate k_4^* and hence the reverse constant k_3^*, which enters into the calculation of CMR_{Glu}. The equation for the calculation of CMR_{Glu} indicates that the rate constants are themselves interrelated and interdependent so that errors in the estimation of individual constants are substantially minimized in the final calculation of CMR_{Glu} (Sokoloff *et al.*, 1977). This is clear on inspection of the standard deviations of the rate constants and a comparison with the much smaller standard error of 6.2% for the derived function $k_1^* k_3^*/(k_2^* + k_3^*)$. The kinetic constants in Table 6 are sufficiently similar between the 3 groups that the alteration in MR would be very unlikely to result from major changes in the true LC which might be brought about by altered handling of ^{18}FDG and glucose at the blood-brain barrier or the hexokinase step as a result of the disease process itself.

The heterogeneity of clinical features in patients with similar underlying biochemical defects is puzzling. The major changes in energy metabolism we have demonstrated would support the hypothesis that the distribution of the biochemical abnormality is not uniform in all the tissues of the body. Thus patients with CNS disease have evidence of disturbed cerebral energy metabolism, whereas those without CNS signs or symptoms do not. This might be explained by dysfunction of tissue specific isoforms of the enzymes or alternatively by a difference in dose and segregation of defective mitochondria during early embryogenesis, if the mode of transmission of this group of disorders is indeed cytoplasmic and maternal (mitochondrial). It may be possible to define the heterogeneous distribution of biochemical dysfunction in the various body tissues in the future more directly by whole body scanning of affected young children. Alternatively, PET could be used to measure tissue pH using tracers such as $^{11}CO_2$ or ^{11}C-DMO (Syrota *et al.*, 1983; Brooks *et al.*, 1984; Buxton *et al.*, 1984, 1986; and *see* Berkovic *et al.*, 1987).

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FAMILIAL MYOPATHY WITH CHANGES RESEMBLING INCLUSION BODY MYOSITIS AND PERIVENTRICULAR LEUCOENCEPHALOPATHY

A NEW SYNDROME

by ANDREW J. COLE,[1] RUBEN KUZNIECKY, GEORGE KARPATI, STIRLING CARPENTER, EVA ANDERMANN *and* FREDERICK ANDERMANN

(*From the Montreal Neurological Hospital and Institute, Montreal, Canada*)

SUMMARY

Five of 6 male siblings were affected by a progressive myopathy beginning in early childhood. Muscle biopsies in all patients showed the characteristic changes of inclusion body myositis. Computerized tomography and magnetic resonance imaging revealed a markedly abnormal appearance of cerebral white matter in the 4 affected patients tested, but clinical and other laboratory examinations failed to demonstrate evidence of central white matter dysfunction. Muscle biopsies and brain imaging were normal in all clinically unaffected family members. On the basis of the genetics, muscle biopsy findings and cerebral white matter changes, we conclude that this constellation represents a hitherto undescribed syndrome.

INTRODUCTION

Inclusion body myositis (IBM) is a well recognized variety of idiopathic inflammatory myopathy characterized by slowly progressive weakness, usually starting in the sixth or seventh decade, and predominantly affecting men. The diagnosis can be suspected on clinical grounds, but can be confirmed only by the finding of typical inclusions in muscle fibres consisting of 15 to 18 nm filaments (Carpenter *et al.*, 1978). A number of reports have described the pathological findings of IBM in younger patients (Sato *et al.*, 1969; Yunis and Samaha, 1971; Tomé *et al.*, 1981; Eisen *et al.*, 1983) and several examples of apparent familial IBM have been reported (Fukuhara *et al.*, 1982; Matsubara and Tanabe, 1982; Eisen *et al.*, 1983).

We investigated a family of 6 male siblings, 5 of whom were affected by a progressive myopathy. In each patient, muscle biopsy demonstrated the character-

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Correspondence to: Dr G. Karpati, Montreal Neurological Hospital and Institute, 3801 University Street, Montreal, Quebec H3A 2B4, Canada.

[1] Present address Johns Hopkins University School of Medicine, Department of Neuroscience, 725 North Wolfe Street, Baltimore, Maryland, 21205, USA.

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istic findings of IBM by light and electron microscopy. Each of the affected patients also showed a striking but asymptomatic abnormality of cerebral white matter on magnetic resonance imaging (MRI).

CASE REPORTS

The familial incidence of this disease is illustrated in fig. 1.

Case II-2

A 35-yr-old man, the first of twins, was born 1 wk before term. He walked at 18 months and was never able to run. Gowers' sign was present from the time he began to stand. Muscle weakness, mainly proximal, progressed slowly during adolescence. Following a motor vehicle accident in which he sustained a femoral fracture at the age of 26 yrs, he required canes to walk.

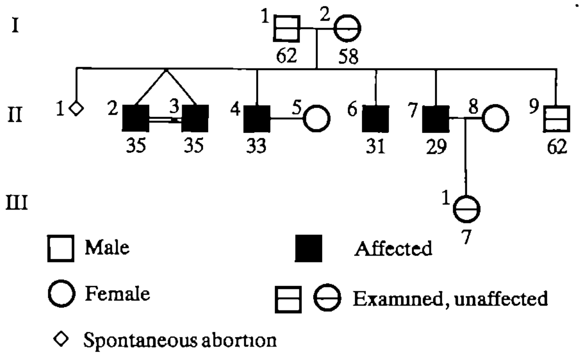

FIG. 1 Pedigree of Family D Proband is II-6.

At 35 yrs he showed an exaggerated lumbar lordosis and bilateral genu recurvatum. He had moderate proximal weakness in all 4 limbs with minimal wasting. His calves were slightly enlarged but had normal texture. Distal muscle strength was normal. Tendon jerks were absent in the arms, reduced at the knees and preserved at the ankles. The plantar responses were flexor. He required crutches to walk, and had a waddling gait. Mentation, cranial nerves, sensation and coordination were intact.

Case II-3

This man was the second-born identical twin of Case II-2. His motor milestones were delayed in spite of normal intellectual development. Muscle weakness, apparent from early childhood, was slowly progressive, and he currently requires one cane to walk.

At 35 yrs he showed mild proximal muscle weakness in the upper limbs, with moderate proximal weakness in the legs. Distal muscles were of normal strength. Calf bulk was slightly increased. Tendon jerks were absent except at the ankles. He had a waddling gait with increased lumbar lordosis. Sensation and coordination were normal.

Case II-4

A 33-yr-old man had been delivered at term following a normal gestation. Motor milestones were delayed: he walked unassisted at 20 months. Intellectual function was normal. He was never able to run. Muscle weakness progressed slowly throughout his life so that by the age of 30 yrs he had difficulty ascending stairs.

At 33 yrs he showed moderate weakness of proximal and distal muscles of the lower limbs but relative sparing of the intrinsic muscles of the feet. Mild shoulder girdle and proximal arm weakness was present with normal hand strength. Craniobulbar musculature was normal. Tendon jerks were absent except at the ankles. Sensation and mentation were normal. Gait was moderately impaired.

Case II-6

The proband, a 31-yr-old man, first walked unassisted at 18 months of age. Intellectual development was normal He had difficulty in school athletics, and muscle weakness continued to progress until the present time. In addition he has suffered from complex partial seizures and occasional generalized convulsions since the age of 7 yrs.

Examination at 31 yrs of age showed moderate kyphoscoliosis Mentation was normal. A right exotropia was present as were bilateral cataracts and retinal scars considered by a neuro-ophthalmologist to be traumatic in origin. Muscle bulk was diminished in association with diffuse weakness which was worse in proximal muscles of all 4 limbs. Ankle jerks were preserved. Gowers' sign was present and his gait was mildly impaired. Sensation was normal.

Case II-7

A 29-yr-old man was the product of a normal gestation and delivery. Intellectual development was normal, but motor milestones were markedly delayed. He was able to sit unassisted at the age of 13 months, and to walk at the age of 24 months He was unable to participate in school athletics because of muscle weakness. There was steady progression of muscle weakness so that by the age of 28 yrs he had great difficulty going up and down stairs. There were no cramps, muscle pains, or fasciculations.

At 29 yrs of age he showed mild kyphoscoliosis and exaggerated lumbar lordosis (fig. 2). The gait was waddling Gowers' sign was present. Muscle bulk was diminished throughout, especially in the upper limbs. There was moderate diffuse weakness which was most apparent in the proximal muscles of the upper and lower limbs. Neck flexors were also weak, and there was winging of the scapulae; intrinsic

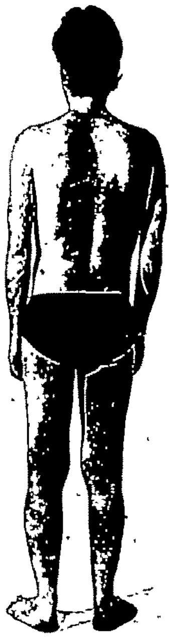

FIG. 2 *Case II-7*. Note patchy wasting of arm and leg musculature, including muscles of the shoulder girdle

hand and foot muscles were well preserved. Tendon reflexes were absent except ankle jerks. The plantar responses were flexor. The mental state, cranial nerves, cerebellar and sensory examinations were normal.

RESULTS

Results of laboratory investigations of patients are presented in Table 1. All tests in unaffected family members were normal or negative.

TABLE 1 SUMMARY OF LABORATORY EXAMINATIONS IN FAMILY D*

Test	*II-2*	*II-3*	*II-4*	*II-6*	*II-7*
CK(IU/dl) (normal range 5–140)	404	391	668	848	737
EMG	Small polyphasic motor units, fibrillation, positive sharp waves, bizarre high frequency discharges, hyperrecruitment				
NCV	Minimal slowing of motor nerve conduction velocities with slight prolongation of distal F wave latencies				
CSF					
Protein (g/l)	0·61	0·68	NA	0·56	NA
Oligoclonal IgG bands	Neg	Neg	NA	Neg	NA
VLCFA	N	N	N	NA	NA
VEP	N	N	N	N	N
BAEP	N	N	N	N	N

* All tests in unaffected family members were normal. CK = serum creatine kinase. EMG = electromyography NCV = nerve conduction velocity NA = not available. Neg = negative. N = normal. VEP = visual evoked potential. BAEP = brainstem auditory evoked potential VLCFA = serum very long chain fatty acids.

Muscle biopsy

Biopsies of biceps brachii muscle from all family members except Case III-1 were processed for histochemical and electron microscopic examination in the routine manner (Carpenter and Karpati, 1984). Each of the affected patients demonstrated the characteristic findings of IBM on histochemical, phase and electron microscopic examination of muscle biopsies (Carpenter *et al.*, 1978) (Table 2). Histochemical study of cryostat sections revealed many abnormally small calibre fibres mixed with some abnormally large fibres. Centrally situated myonuclei were increased in number. There was a moderate increase in endomysial connective tissue. Rimmed vacuoles were present in many muscle fibres throughout the specimens (fig. 3). Some rimmed vacuoles contained impressive eosinophylic inclusions (fig. 4A). In a few

TABLE 2 SUMMARY OF PATHOLOGICAL FINDINGS IN MUSCLE IN FAMILY D

	Case				
	II-2	*II-3*	*II-4*	*II-6*	*II-7*
Age (yrs)	34	34	32	29	28
Muscle fibre loss	+ + +	+ + +	+ + +	+	+
Necrosis	+ +	+	+	0	+
Rimmed vacuoles	+ + +	+ +	+	+ + +	+ + +
Variability of fibre calibre	+ + +	+ + +	+ + +	+ + +	+ + +
Abnormal 15–18 nm cytoplasmic filamentous inclusions observed	Y	Y	Y	Y	N

0 = absent + = mild. + + = moderate + + + = severe. Y = yes N = no

fibres, the chromatin of some nuclei was displaced to the periphery of the nucleus by masses of inclusion material (fig. 4B). In the most severely affected patients (II-2, II-3, II-4), massive replacement of muscle fibres with adipose tissue was seen (fig. 5). Phase microscopy on resin sections showed dark granules in several muscle fibres in all biopsies (fig. 6), except that of Case II-7, where there were very few fibres. Nuclear abnormalities were not observed. Electron microscopy showed membranous whorls and accumulations of characteristic 15 to 18 nm filaments in the cytoplasm but not in nuclei of muscle fibres (fig. 7). The mother (I-2) and the unaffected brother (II-9) had

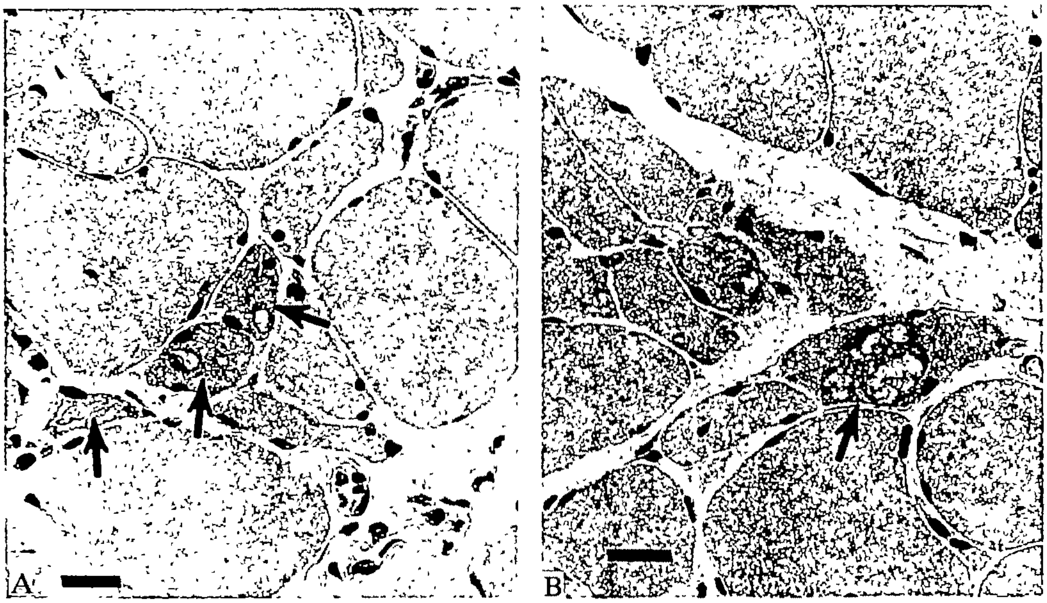

FIG. 3. Biopsy of *Case II-7*. A, arrows mark 3 fibres with prominent rimmed vacuoles B, a fibre in the centre (arrow) is overwhelmed by rimmed vacuoles. Haematoxylin and eosin Bar = 25 μm

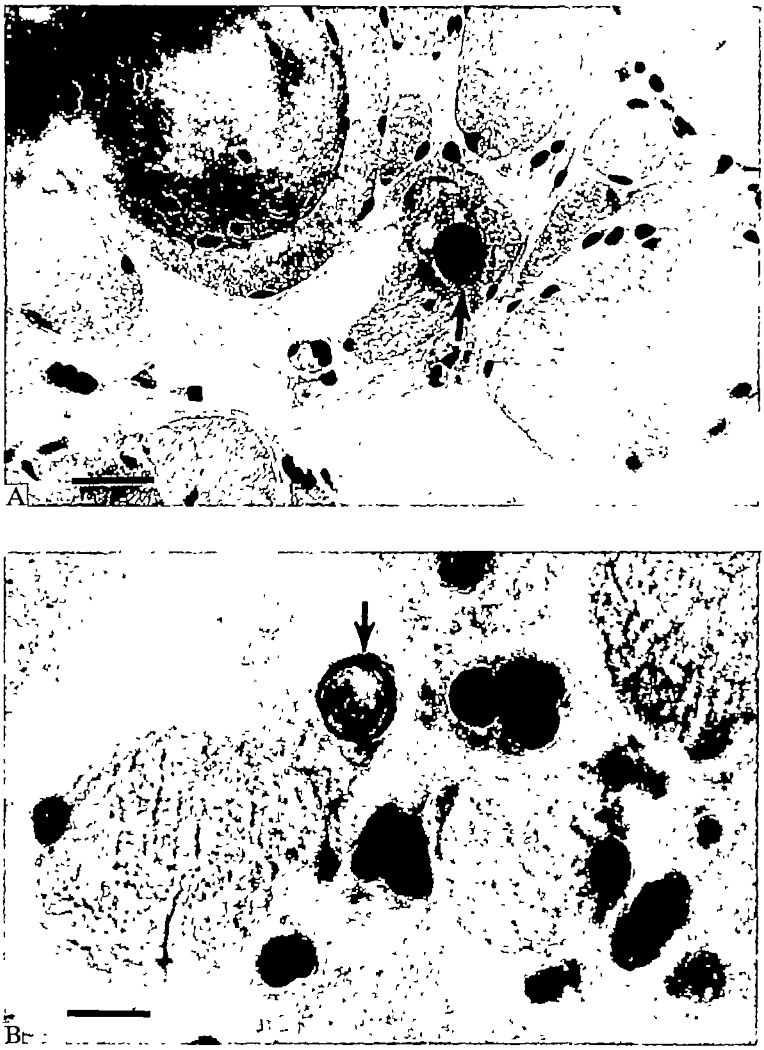

FIG. 4. *Case II-7.* A, muscle biopsy. A massive eosinophilic inclusion body is present in a large rimmed vacuole (arrow). In the left upper corner, a massively hypertrophied muscle fibre shows spatial disorientation of myofibrils. Haematoxylin and eosin. Bar = 25 μm. B, muscle biopsy. In an otherwise normal muscle fibre, a nucleus (arrow) is greatly enlarged by material that displaces the nuclear chromatin to the periphery. Haematoxylin and eosin. Bar = 10 μm

normal biopsies. The father (I-1), who was entirely asymptomatic, had a number of tubular aggregates in his biceps biopsy. Intramuscular nerves in each case were normal.

Brain imaging

Cranial CT scanning was performed in 4 of the affected patients (II-2, II-3, II-6, II-7) and showed hypodensity of cerebral white matter (fig. 8). MRI of the brain in all

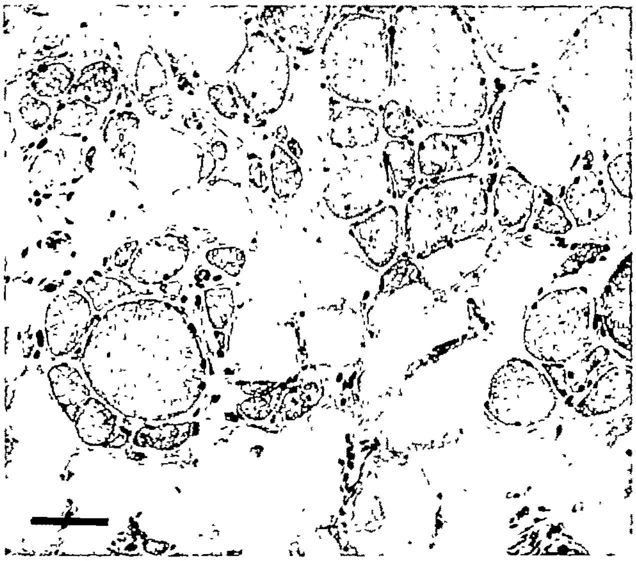

FIG 5. Biopsy of *Case II-2* There is prominent loss of muscle fibres with replacement by adipocytes. Most of the remaining fibres are abnormally small or large in calibre. Haematoxylin and eosin. Bar = 100 μm

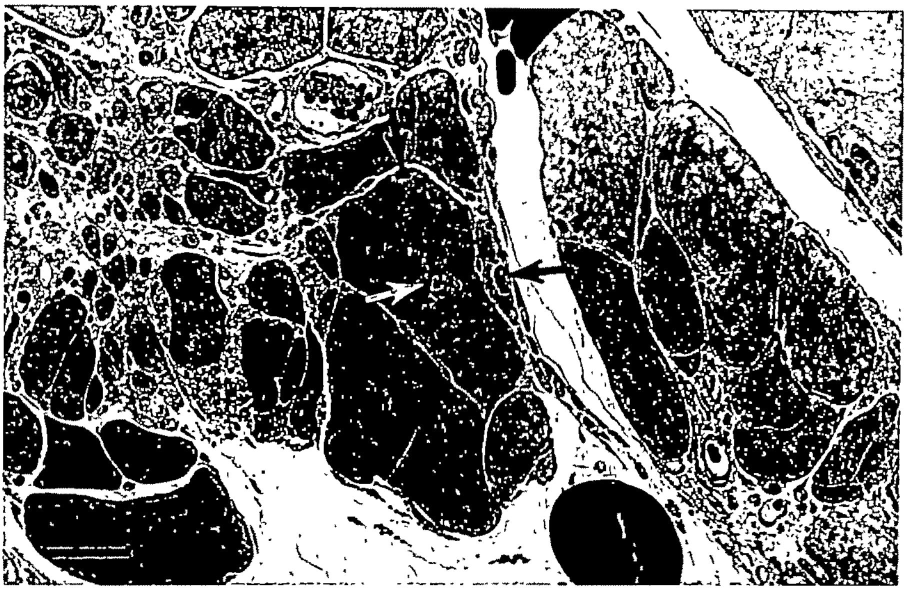

FIG 6. Biopsy of *Case II-6*. Dark arrows point to membranous whorls, white arrow to a cytoplasmic inclusion bordered by membranous whorls. Note the marked variation in fibre size and collagen deposition between fibres Resin section, paraphenylene diamine. Bar = 50 μm.

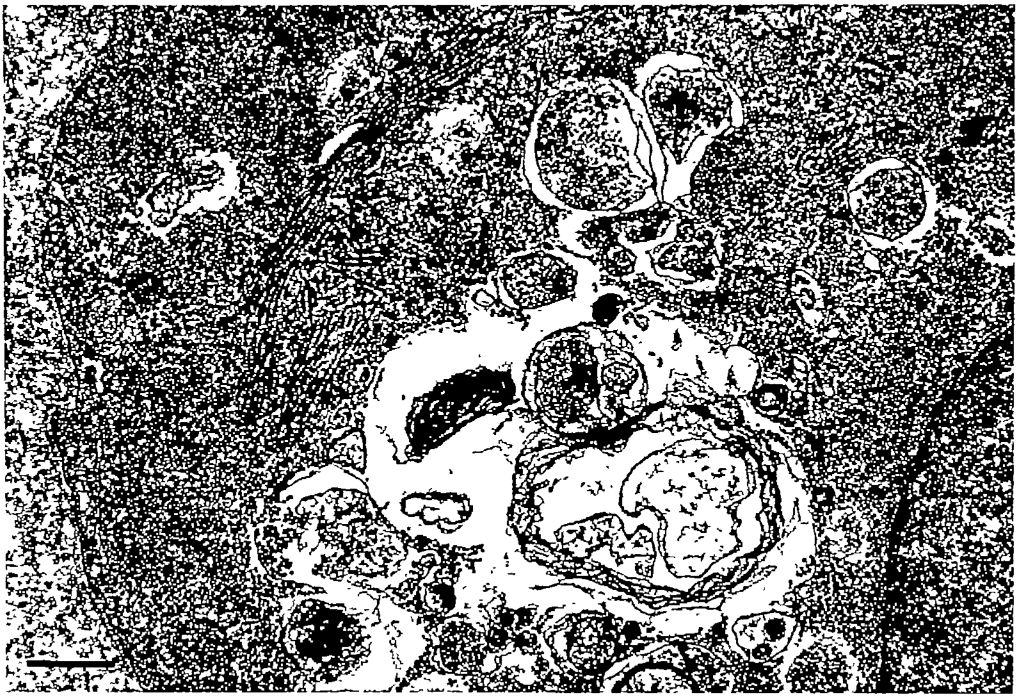

FIG 7. Biopsy of *Case II-6*. This electron micrograph shows a collection of abnormal filaments (arrow) in a muscle fibre near some membranous whorls. Bar = 0.5 μm.

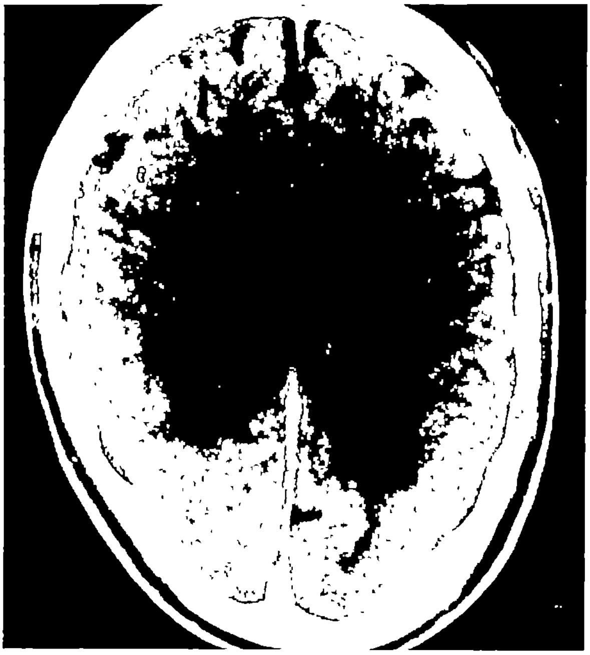

FIG 8 Contrast-enhanced head CT scan from *Case II-6* Note periventricular hypodensity with accentuation in the region of the frontal and occipital horns.

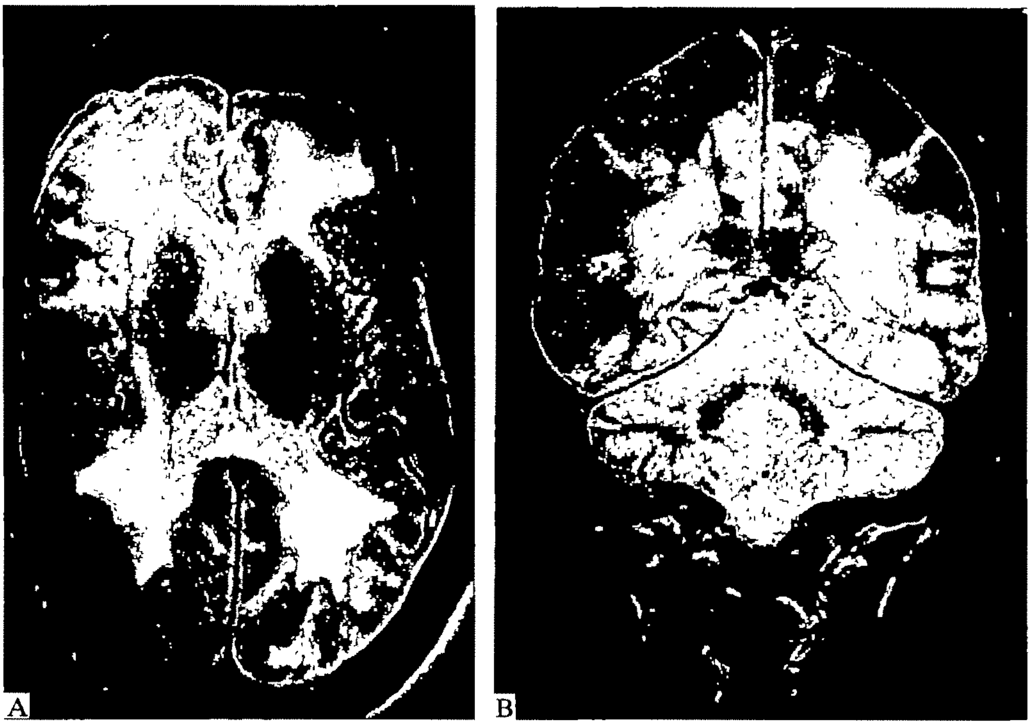

FIG. 9. A, magnetic resonance imaging of brain from *Case II-2*, axial slice. TR = 2100 ms, TE = 60 ms. The cerebral white matter is conspicuous by abnormally high intensity signal B, magnetic resonance imaging of brain, from *Case II-2*, coronal cut TR = 2100 ms, TE = 60 ms. The abnormally high intensity signal of the cerebral white matter appears to spare the subcortical U fibres

affected brothers showed strikingly increased signal intensity from cerebral white matter, including periventricular white matter and the corona radiata, but sparing the subcortical U-fibre system (fig. 9). In 2 cases (II-6, II-7), the increased signal intensity took on a nodular appearance. The severity of white matter change roughly correlated with the severity of myopathy in affected patients. Imaging studies in all unaffected family members were normal.

Additional studies

A 68-yr-old man with pathologically documented typical sporadic IBM was examined with MRI: his cerebral white matter was entirely normal. An unrelated 71-yr-old woman with well documented IBM died of respiratory failure and had a complete postmortem examination. No abnormality of cerebral white matter was detected on gross or microscopic examination. An Iranian-Jewish woman with a progressive myopathy beginning in the third decade, who had a similarly affected brother, demonstrated the typical pathological changes of IBM on muscle biopsy. A brain MRI scan in that patient was normal.

DISCUSSION

Our patients presented with a progressive myopathy associated with evidence of abnormal cerebral white matter on brain imaging. Since the pathological features of the myopathy were indistinguishable from those of inclusion body myositis (IBM), but the clinical picture was atypical for that disease, we shall compare our cases to those in the literature with typical and with atypical forms of IBM. Furthermore, we shall discuss the relationship of the cerebral white matter changes to the syndrome.

The pathological changes found in muscle biopsies in our patients are entirely consistent with the diagnosis of IBM. Variations in fibre calibre, rimmed vacuoles, proliferation of endomysial connective tissue, membranous whorls and inclusions composed of 15 to 18 nm filaments are all characteristic, and in combination have been considered pathognomonic of that disorder. While inflammatory infiltrates may be present in typical IBM, they are not required for diagnosis (Carpenter and Karpati, 1981, 1984) and were absent in our patients. The typical filamentous nuclear or cytoplasmic inclusions present in these patients' biopsies are almost exclusively seen in classical IBM. In a few recent reports, these filaments have been described in association with neuromuscular diseases variously labelled as oculopharyngeal muscular dystrophy and distal myopathy (Fukuhara *et al.*, 1982; Matsubara and Tanabe, 1982; Smith and Chad, 1984). Whether these myopathies are distinct from IBM, or whether they represent variants of IBM cannot presently be determined. Thus the disease specificity of the 15 to 18 nm filaments remains uncertain.

The clinical features of the myopathy observed in these patients differ in a number of significant respects from the usual presentation of IBM. IBM most often begins in the sixth or seventh decade of life. While cases have been described beginning as early as the second decade (Sato *et al.*, 1969; Yunis and Samaha, 1971; Jerusalem *et al.*, 1972; Tomé *et al.*, 1981), onset in early childhood would be extraordinary and has been reported only in 1 case by Eisen *et al.* (1983). Weakness in classical IBM may involve all muscle groups, but is often predominant distally (Carpenter *et al.*, 1978), in contrast to the present cases where weakness was mainly proximal.

A number of reports have described familial myopathies that fulfil the pathological criteria required for a diagnosis of IBM. Eisen *et al.* (1983) described a 23-yr-old man with a slowly progressive distal myopathy beginning in early childhood whose muscle biopsy revealed the typical findings of IBM. That patient had a brother affected with a similar illness who was not studied. Matsubara and Tanabe (1982) reported a brother and sister affected by a progressive distal myopathy during the third decade who also met the biopsy criteria for a diagnosis of IBM. They characterized this disease as an 'autosomal recessive distal myopathy'. Fukuhara *et al.* (1982) described a 39-yr-old man with the clinical picture of 'oculopharyngeal muscular dystrophy' (OMD) that began at the age of 23 yrs with ptosis and proximal muscle wasting. That patient's son was noted to be clumsy at 2 yrs of age, and developed leg weakness and wasting at the age of 6 yrs. Facial weakness with pronounced distal weakness led to muscle biopsy at 9 yrs. Muscle biopsies in each of these patients showed rimmed vacuoles. Electron microscopy was reported in the second patient only, and demonstrated cytoplasmic inclusions associated with 13 to 19 nm filaments. These authors speculated on the relationship between OMD and distal myopathy and suggested that there might be an aetiological link between the two disorders. They concluded that 'distal myopathy', OMD and IBM were variant forms of the same disease. Argov and Yarom (1984) reported 9 examples of a generalized myopathy sparing the quadriceps in Iranian Jews beginning in the third or fourth decade. Biopsies were characterized by the presence of rimmed vacuoles in affected muscles. Abnormal filaments were not mentioned in the description of electron microscopic findings. Inheritance was felt to be autosomal recessive. Our finding of typical 15 to 18 nm filamentous inclusions in the muscle biopsy from an Iranian-Jewish woman with a familial myopathy beginning during the third decade suggests that this population may indeed suffer from a form of familial IBM. Detailed neuroimaging studies were not described in any of these reports of familial IBM. The Iranian-Jewish woman whom we studied had a normal MRI brain scan, confirming that changes in cerebral white matter are not characteristic of all familial examples of IBM.

The family that is the subject of the present report differs significantly from each of these reported examples of 'familial IBM'. Our patients were all affected early in life, and all demonstrated mainly proximal weakness. Because of the absence of female siblings, as well as the occurrence of this syndrome in only a single sibship, the mode of inheritance cannot be determined with certainty. The absence of female siblings makes it impossible to differentiate between autosomal recessive or X-linked recessive inheritance. The absence of muscle biopsy findings of IBM or of white matter abnormality in either of the parents argues strongly against the possibility of dominant transmission, although incomplete penetrance of the trait or a mutation in either of the parental germ-cell lines cannot be totally eliminated. Maternal inheritance, described in certain mitochondrial cytopathies (Giles *et al.*, 1980; Egger and Wilson, 1983; Rosing *et al.*, 1985) cannot be eliminated in view of the marked phenotypic variability from one generation to the next in those disorders (DiMauro *et al.*, 1985). There are numerous examples of neurological diseases that apparently occur in both familial and sporadic forms, such as Creutzfeld-Jakob disease, Alzheimer's disease, and amyotrophic lateral sclerosis. In each of these diseases hypotheses of vertical transmission of an infectious agent, genetic susceptibility to an environmental agent, and similarity of phenotypic expression of different disease processes have been suggested. Each of these hypotheses could explain the relationship between familial and sporadic IBM. Evidence that IBM is caused by a persistent mumps virus infection (Chou, 1986) has not yet been confirmed.

The white matter hypodensity on CT scans, and the high signal intensity from white matter seen on MRI in these patients appeared as severe in degree as that seen in cases of leucodystrophy, yet the patients had no symptoms of white matter dysfunction. A number of myopathies have been shown to be associated with abnormal CNS function and/or appearance. It has long been recognized that Duchenne muscular dystrophy and myotonic dystrophy may be associated with mild or moderate mental retardation, and with abnormalities of cerebral cortical architecture (Rosman and Kakulas, 1966). Several reports have emphasized the association of certain congenital muscular dystrophies with CNS changes (Bernier *et al.*, 1979; Nogen, 1980; Fukuyama *et al.*, 1981; Egger *et al.*, 1983; Echenne *et al.*, 1986) which may range from apparently asymptomatic hypodensity of cerebral white matter on CT scanning to widespread abnormality of cerebral architecture involving grey and/or white matter. A number of putative mitochondrial cytopathies have also been associated with CNS white matter abnormalities, for example the Kearns-Sayre-Shy syndrome of progressive external ophthalmoplegia. The clinical picture in our family, however, is inconsistent with any of these diagnoses. Nevertheless, the correlation between severity of white matter change and both clinical and pathological severity of myopathy in our patients suggests that the white matter change and the muscle disease may be related to the same pathogenetic factor. We cannot, however, rule out the possibility that these changes are only related by genetic linkage; completely coincidental occurrence seems unlikely.

The disease described in this report appears to be unique. The familial occurrence of a slowly progressive myopathy beginning in early childhood meeting the pathological criteria for a diagnosis of IBM, associated with a distinctive abnormality of cerebral white matter, suggests that this is a newly recognized syndrome. This may indicate either that the clinical spectrum of IBM is wider than originally thought, or that the pathological changes of IBM are nonspecific and may be found in other disorders. To put it differently, not all progressive muscle diseases with 15 to 18 nm filaments necessarily represent IBM. Only the identification of an underlying biochemical, molecular or immunological defect would allow clarification of the relationship between the disease occurring in our family and cases of typical IBM.

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THE ROLE OF MACROPHAGES AND EICOSANOIDS IN THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC NEURITIS

SERIAL CLINICAL, ELECTROPHYSIOLOGICAL, BIOCHEMICAL AND MORPHOLOGICAL OBSERVATIONS

by HANS-PETER HARTUNG, BÄRBEL SCHÄFER, KURT HEININGER, GUIDO STOLL *and* KLAUS V. TOYKA

(From the Department of Neurology, University of Düsseldorf, FRG)

SUMMARY

Experimental allergic neuritis (EAN) can be prevented or ameliorated by global blockade of macrophages. How these cells damage peripheral nervous tissue in this autoimmune demyelinating polyneuropathy is not fully understood. Since macrophages exert a number of their inflammatory actions by the release of arachidonic acid-derived eicosanoids, we investigated the possible role of these mediators in the pathogenesis of EAN. Lewis rats with myelin-induced EAN were treated before and after onset of clinical signs. Administration of corticosteroids or of the cyclo-oxygenase inhibitors indomethacin and BW755c before the onset of neurological signs suppressed the disease, as judged by clinical assessment, serial electrophysiological testing and histological examination, while initiation of drug treatment on day 13 postimmunization still markedly attenuated the course of EAN. The selective lipoxygenase blocker nafazatrom had only a slight effect. Determination of the production by macrophages ex vivo of eicosanoids corroborated the predicted site of action of the pharmacological compounds applied. We infer that macrophage-derived proinflammatory arachidonic acid metabolites significantly contribute to functional and tissue damage in EAN. Our results may be relevant to future pharmacological treatment of the acute Guillain-Barré syndrome.

INTRODUCTION

Experimental allergic neuritis (EAN) is an inflammatory demyelinating polyradiculoneuropathy that can be produced in a number of species by immunization with whole peripheral nervous tissue homogenates (Waksman and Adams, 1955), myelin (Smith *et al.*, 1979), a purified neuritogenic component of myelin, the P_2 protein (Brostoff *et al.*, 1977; Kadlubowski and Hughes, 1979), or by adoptive transfer of P_2-specific cloned T lymphocytes upon naive Lewis rats (Linington *et al.*, 1984). On clinical, neurophysiological and pathological grounds, EAN is considered to represent an animal model of the acute Guillain-Barré syndrome (Leibowitz and

Correspondence: Dr H.-P. Hartung, Department of Neurology, University of Düsseldorf, Moorenstrasse 5, D-4000 Düsseldorf, FRG.

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Hughes, 1983). EAN presents clinically with impaired righting and limp tail at the onset, followed by increasing ataxic weakness, and progresses in a small proportion of cases to quadriplegia and death. Both humoral and cell-mediated immune responses have been implicated in its pathogenesis (Saida *et al.*, 1979; Hughes *et al.*, 1981; Leibowitz and Hughes, 1983; Linington *et al.*, 1984; Toyka and Heininger, 1987; Hartung *et al.*, 1988).

Pathologically, EAN is characterized by the infiltration of the peripheral nervous system by lymphocytes and macrophages (Waksman and Adams, 1955; Lampert, 1969; Raine, 1985). As the inflammatory lesion proceeds, macrophages emerge as the predominant cells. Electron microscope studies have revealed that these cells actively strip off myelin lamellae from axons, induce vesicular disruption, and phagocytose both intact and damaged myelin (Ballin and Thomas, 1969; Lampert, 1969). Global blockade of macrophage functions achieved with administration of silica in vivo, suppresses or attenuates the disease in Lewis rats (Tansey and Brosnan, 1982; Craggs *et al.*, 1984).

Since macrophages exert many of their functions through generation and release of arachidonic acid-derived mediators such as prostaglandins and leukotrienes (Gemsa, 1981; Adams and Hamilton, 1984; Bonney and Humes, 1984) we studied the effects of known inhibitors of eicosanoid biosynthesis on the clinical, electrophysiological and histological manifestations of EAN elicited in Lewis rats by inoculation with purified bovine root myelin.

MATERIALS AND METHODS

Animals

Inbred female Lewis rats (Zentralinstitut für Versuchstierforschung Hannover, FRG), 10–12 weeks old and weighing 180–200 g, were kept in plastic cages and given standard chow and water ad libitum.

Immunization

PNS myelin was prepared from bovine spinal roots (Norton and Poduslo, 1973). EAN was induced by inoculation with 3 mg myelin in 150 μl saline emulsified with an equal volume of complete Freund's adjuvant containing 20 mg/ml Mycobacterium tuberculosis (OREC 20/21, Behring, Marburg). Antigen plus adjuvant was injected into 3 footpads sparing the left hindlimb to be used for electrophysiological recordings, and into the neck region.

Clinical assessment

The severity of the disease was assessed clinically, employing the scale devised by King *et al.* (1983), on which animals are given scores ranging from 0–10 (0 = normal, 1 = less lively, 2 = impaired righting/limp tail, 3 = absent righting, 4 = ataxic gait, abnormal position, 5 = mild paraparesis, 6 = moderate paraparesis, 7 = severe paraparesis, 8 = tetraparesis, 9 = moribund, 10 = death). Scoring was performed independently by 2 observers who were masked with regard to allocation of animals to one of the various treatment groups.

Electrophysiological tests

Rats were anaesthetized with 1 ml/kg body weight Hypnorm (Janssen Pharmaceutical, Beerse, Belgium) containing 10 mg/ml fluanisone and 0.315 mg/ml fentanyldihydrogen citrate. This neuroleptic-analgesic combination does not affect peripheral or central nerve conduction (Heininger *et al.*, 1986). Electrophysiological recordings were performed blindly as described previously (Heininger *et al.*, 1986) employing a Medelec MS 91a electromyograph, the electrophysiologist being unaware of the clinical status of the animal before anaesthesia. Before immunization and on days 1, 4, 7, 13, 17, 19 and 21 post immunization (p.i.), amplitudes of compound muscle action potentials (CMAPs) evoked by proximal and distal stimulation of the left sciatic nerve, distal F wave latencies, motor and mixed afferent nerve conduction velocity (NCV) and latencies of the S response of lumbar somatosensory evoked potentials (SEPs) were measured serially. In order to reduce individual variations of CMAP amplitudes between rats, groups of animals were formed with little difference in this parameter. This seemed appropriate because CMAP amplitudes are influenced by multiple factors other than neurogenic dysfunction (e.g., weight loss), and these may mask small treatment-induced changes.

Collection and culture of macrophages

Two days before they were killed, rats received an intraperitoneal injection of 0.5% albumin to recruit activated monocytes. Immediately before perfusion the peritoneal cavity of the animals was rinsed with Dulbecco's modified minimal essential medium (Gibco, Karlsruhe) supplemented with heparin, and peritoneal cells were purified on plastic tissue culture dishes (Cluster 24, Costar,

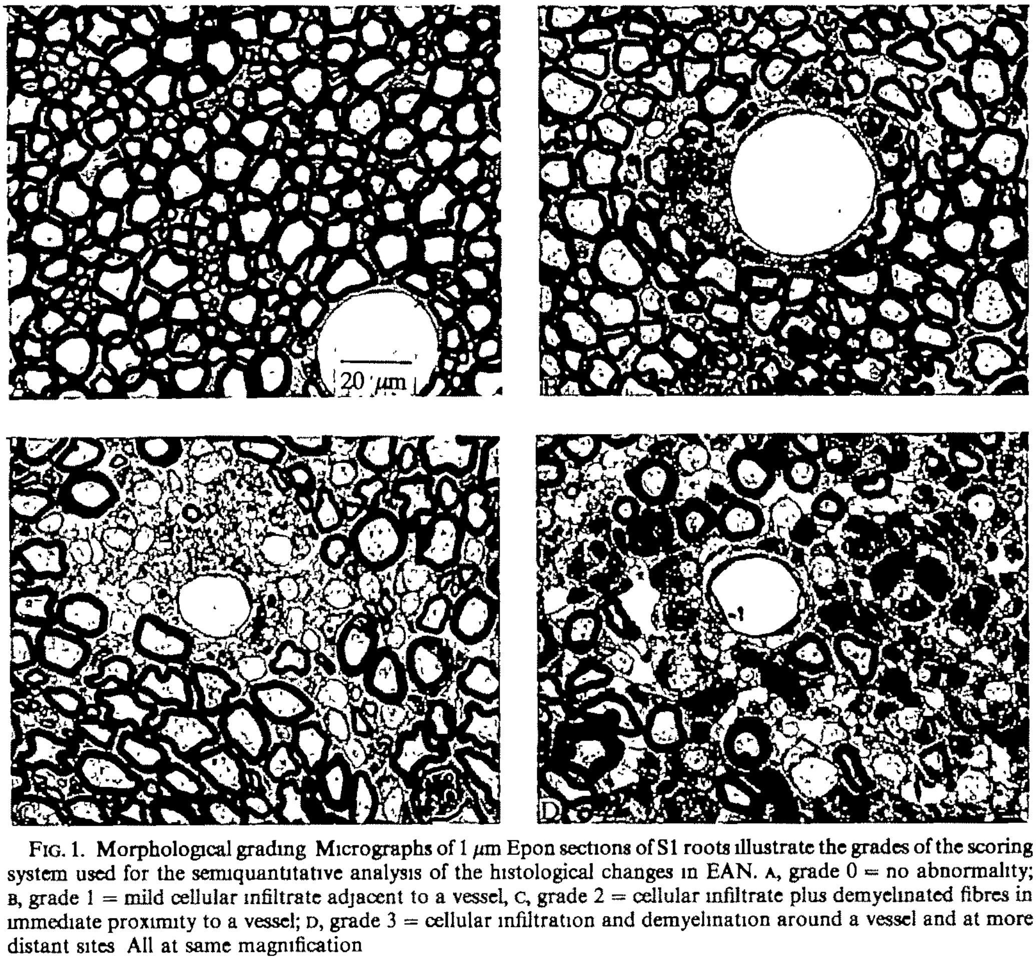


FIG. 1. Morphological grading. Micrographs of 1 µm Epon sections of S1 roots illustrate the grades of the scoring system used for the semiquantitative analysis of the histological changes in EAN. A, grade 0 = no abnormality; B, grade 1 = mild cellular infiltrate adjacent to a vessel, C, grade 2 = cellular infiltrate plus demyelinated fibres in immediate proximity to a vessel; D, grade 3 = cellular infiltration and demyelination around a vessel and at more distant sites. All at same magnification.

Cambridge, USA) (Hartung *et al.*, 1983*a*). Macrophage monolayers of greater than 95% purity, evidenced by phase contrast microscopy, nonspecific esterase staining and phagocytosis of latex particles, were challenged with 40 μg/ml zymasan and kept in short-term culture for 6 h. Cell-free supernatants were stored at −70 °C until radioimmunoassay for prostaglandin E (PGE) or leukotriene C_4 (LTC_4) (Hartung *et al*, 1983*b*, 1986). Results are related to protein content of monolayers using the Lowry method (Lowry *et al.*, 1951).

Histological techniques

Animals were anaesthetized with 30 mg/kg pentobarbital and perfused through the left cardiac ventricle with a 10% dextran solution containing 10,000 U/l heparin followed by a 4% formaldehyde/0.5% glutaraldehyde solution in phosphate-buffered saline Spinal cord with adjacent roots and sciatic nerves were dissected and fixed for an additional 2 h. Specimens were postfixed in 2% osmium tetroxide for 3 h and embedded in Epon. Plastic sections (1 μm) cut at predetermined different levels of the cord, the cauda equina, and the right sciatic nerve, were stained with toluidine blue. Coded sections from the root level were examined by two masked observers and evaluated semiquantitatively (Heininger *et al.*, 1988). A total of 250 perivascular areas of each animal were assessed, and histological changes were graded in severity on the following scale: 0 = normal perivascular area, 1 = mild cellular infiltrate adjacent to a vessel, 2 = cellular infiltrate plus demyelinated fibres in immediate proximity to a vessel, 3 = cellular infiltrate plus demyelination around a vessel and at more distant sites (fig. 1). The total percentage of lesion sites belonging to any one of these categories was estimated for each treatment and control group.

For immunocytochemical studies 10 μm sections of sciatic nerves or spinal cord specimens with adjacent roots embedded in paraffin were mounted on slides and deparaffinized. Cellular infiltrates were characterized using the avidin-biotin complex technique (Stoll *et al.*, 1985, 1986) with monoclonal mouse antibody as the primary reagent. The monoclonal antibody ED1 which specifically recognizes rat macrophages (Dijkstra *et al.*, 1985) was kindly supplied by Dr C. Dijkstra, Vrije Universiteit, Amsterdam.

Experimental protocol

Six animals immunized with myelin received repeated intraperitoneal injections of 200 mg silica (5 μm diameter particles, Sigma, München, FRG) in 1 ml of 0.9% saline on days 3, 7, 11, 15 p.i. Other

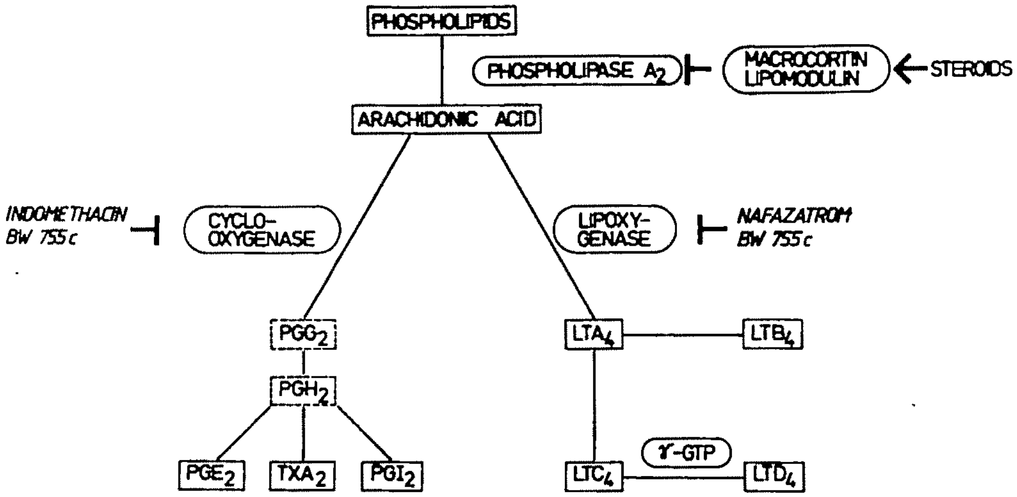


FIG 2. Arachidonic acid metabolism. The pathways of arachidonic acid conversion to eicosanoids are shown and sites identified at which the pharmacological agents that were employed exert an inhibitory action.

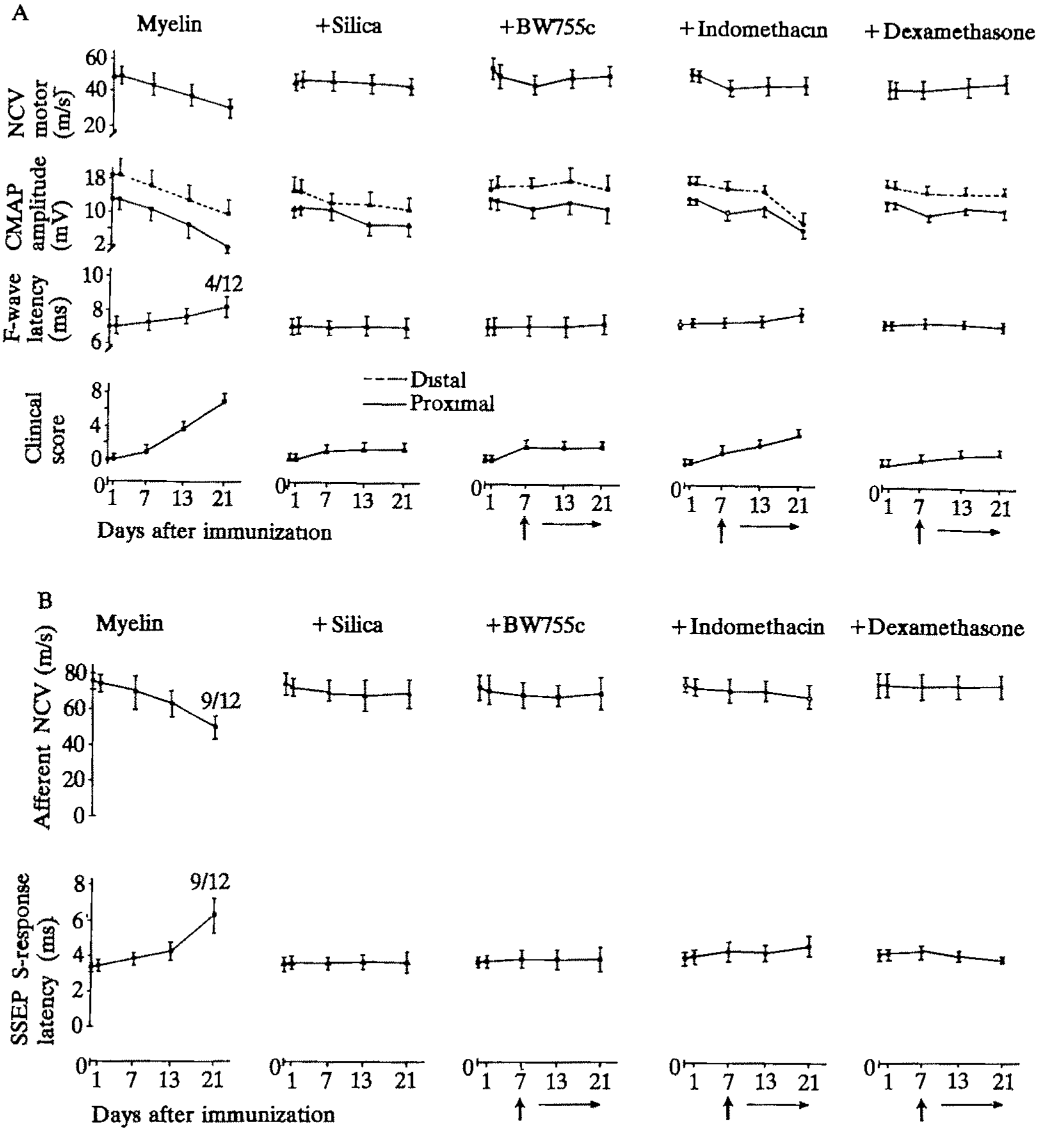


Fig 3. Time course of clinical signs and electrophysiological findings in sham-treated and treated rats with EAN: early treatment. A, motor nerve conduction velocity (NCV) for the sciatic nerve with stimulation at the sciatic notch, amplitude of the evoked compound muscle action potential (CMAP) with stimulation at the lateral malleolus (distal) and at the sciatic notch (proximal). F wave latencies were obtained with ankle stimulation and clinical scoring performed according to King *et al* (1983). B, mixed afferent nerve conduction velocity of the sciatic nerve with stimulation at the malleolus and recording with near-nerve needle electrodes at the sciatic notch, latencies of the S response of lumbar somatosensory evoked potential (SEP) recorded at the T12/L1 spinal level. Myelin designates sham-treated group Bars = SDs. Silica was injected on days 3, 7, 11, 15 p.i. Administration of all other compounds was begun on day 7 p i. An F response could only be obtained in 4 out of 12 sham-treated animals and an S response in 9 out of 12 rats on day 21 p.i. Loss in CMAP amplitude is in part due to temporal dispersion (*see* fig. 4). At the termination of the study, animals that had received treatment (start indicated by vertical arrows), with silica, dexamethasone, indomethacin or BW755c differed significantly ($P < 0.05$) in their electrophysiological parameters from those which had undergone sham treatment.

treatment groups consisted of 6 animals each in which administration of one of the agents given below was initiated on day 7 p.i. to be continued until killing on day 21. In further groups made up of 6 animals each, drug administration was started on day 13 or on day 17, and stopped on day 21. Dexamethasone (Fortecortin, Merck, Darmstadt, FRG) was injected subcutaneously at 4 mg/kg body weight in 2 divided doses. Indomethacin (Sigma, München) (1 mg/kg), BW755c (3-amino-1(m-(trifluoro-methyl-phenyl))-pyrazoline) (Wellcome Research Laboratories, Beckenham, Kent) (50 mg/kg), kindly provided by Dr G. A Higgs, Beckenham, or nafazatrom (1-(2-(β-naphtyloxy)ethyl)-3-methyl-2-pyrazolin-5-one) (50 mg/kg) (Bayer, FRG), donated by Drs K. Brandau and E. Möller, were all applied in 3 divided daily doses via a feeding cannula (fig. 2).

Six animals each were sham-treated either with i.p. injections of 0.9% sterile saline or were fed with drug carrier (0.5% carboxymethylcellulose). Additional groups of 4 animals each received drug treatment from day 13 to day 21, and were then followed clinically and electrophysiologically until killing on day 45.

Statistical evaluation was performed with the nonparametric Mann-Whitney U test. Differences of parameters between groups were considered significant for P values less than 0.05. For these calculations all sham-treated controls were grouped together.

RESULTS

Sham-treated EAN

Animals became less lively (score 1) 7-9 days p.i., showed a limp tail on day 11 and a mild ataxic paraparesis on day 13. Maximal disease severity was reached on day 21 (fig. 3). Progression of the clinical manifestations was paralleled by increasing functional deficits reflected by slowing of motor nerve conduction (approximately 40% mean reduction versus preimmunization), decreased afferent nerve conduction velocity (35% mean reduction), lowering of both proximal and distal amplitudes (which may in part be due to the observed weight loss), temporal dispersion of the CMAP, and eventually conduction block of 80% to 100% (fig. 4).

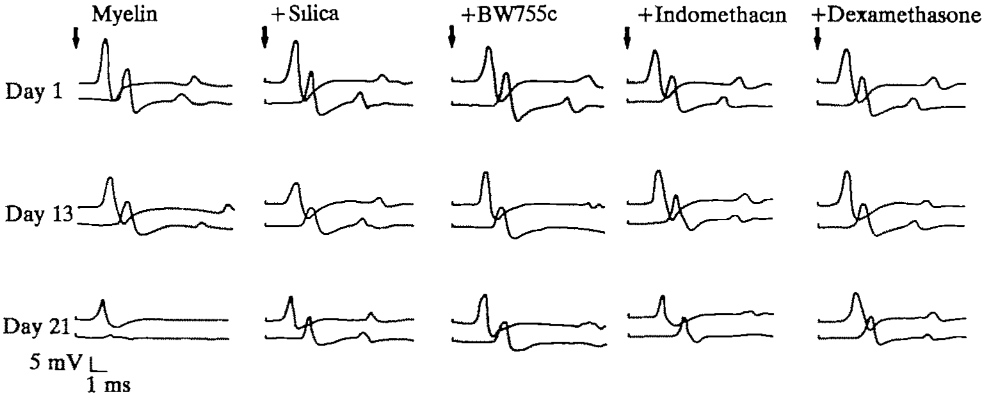


FIG. 4 Serial motor nerve conduction studies. Representative redrawn original recordings of compound muscle action potentials are displayed obtained from the small foot muscles after stimulation of the sciatic nerve distally (*upper traces*) and proximally (*lower traces*) Rats were sham-treated (indicated 'Myelin') or received silica or one of the inhibitors of arachidonic acid metabolism from day 7 to 21 p.i. Note developing failures of F waves, conduction block and slowing with time in sham-treated rats, and only minor changes in treated animals. Arrows indicate stimulus artefact.

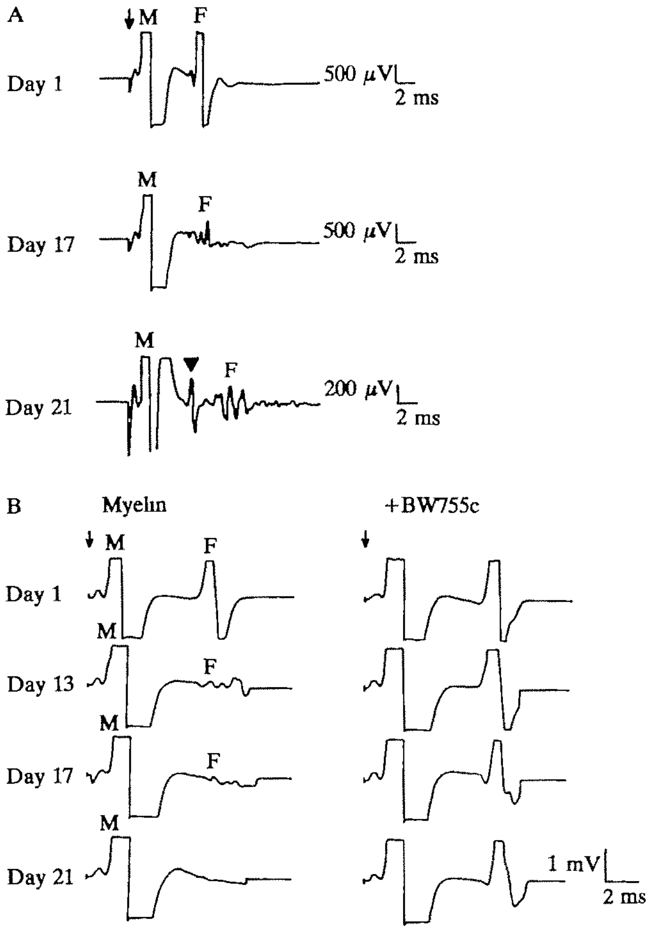


FIG 5 Serial F wave studies in sham-treated and BW755c-treated rats. A, redrawn representative original recordings of F responses obtained after distal stimulation of the sciatic nerve illustrating evolving delays in latency and temporal dispersion In 8 out of 12 animals no F responses could be elicited on day 21 p.i. Arrow = stimulus artefact Note different gains as indicated by the scales. A late potential (marked by triangle) of the M response is seen on day 21 p.i. B, effects of BW755c when administered from day 7 p.i.

F wave latencies were markedly prolonged by day 13 p.i., and F responses became dispersed (fig. 5). On day 21, in only 4 out of 12 animals could an F wave response still be elicited. The S response latency of the lumbar SEP greatly increased from a mean of 3.3+0.5 ms to 6.1+1.1 ms (i.e., up to 185%), its amplitude was reduced, and in 3 animals no response was recordable on day 21 (figs 6, 7).

Effects of silica

Repeated intraperitoneal injections of EAN rats with silica completely suppressed development of the disease judged from clinical assessment and electrophysiological monitoring (figs 3, 4). The only significant abnormality that evolved was a decrease of both proximal and distal amplitudes of the CMAP (mean reduction 20%).

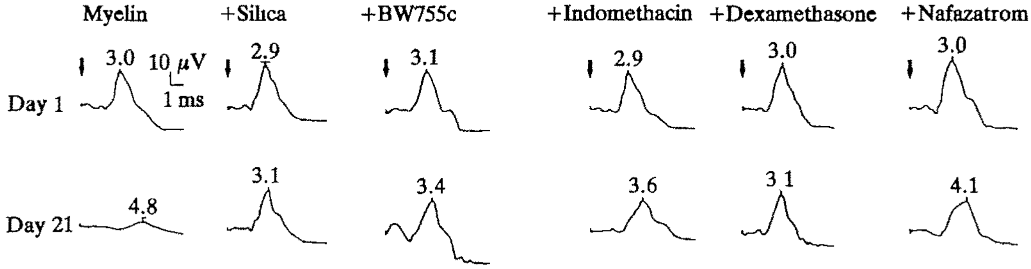


FIG. 6. Lumbar somatosensory evoked potentials The time course of changes of the lumbar SEP recorded at the T12/L1 spinal level is illustrated (representative redrawn original recordings). Treatment was commenced on day 7 p i. Numbers indicate latencies of the S response. In 3 out of 12 sham-treated rats no S response could be obtained. Arrows = stimulus artefact

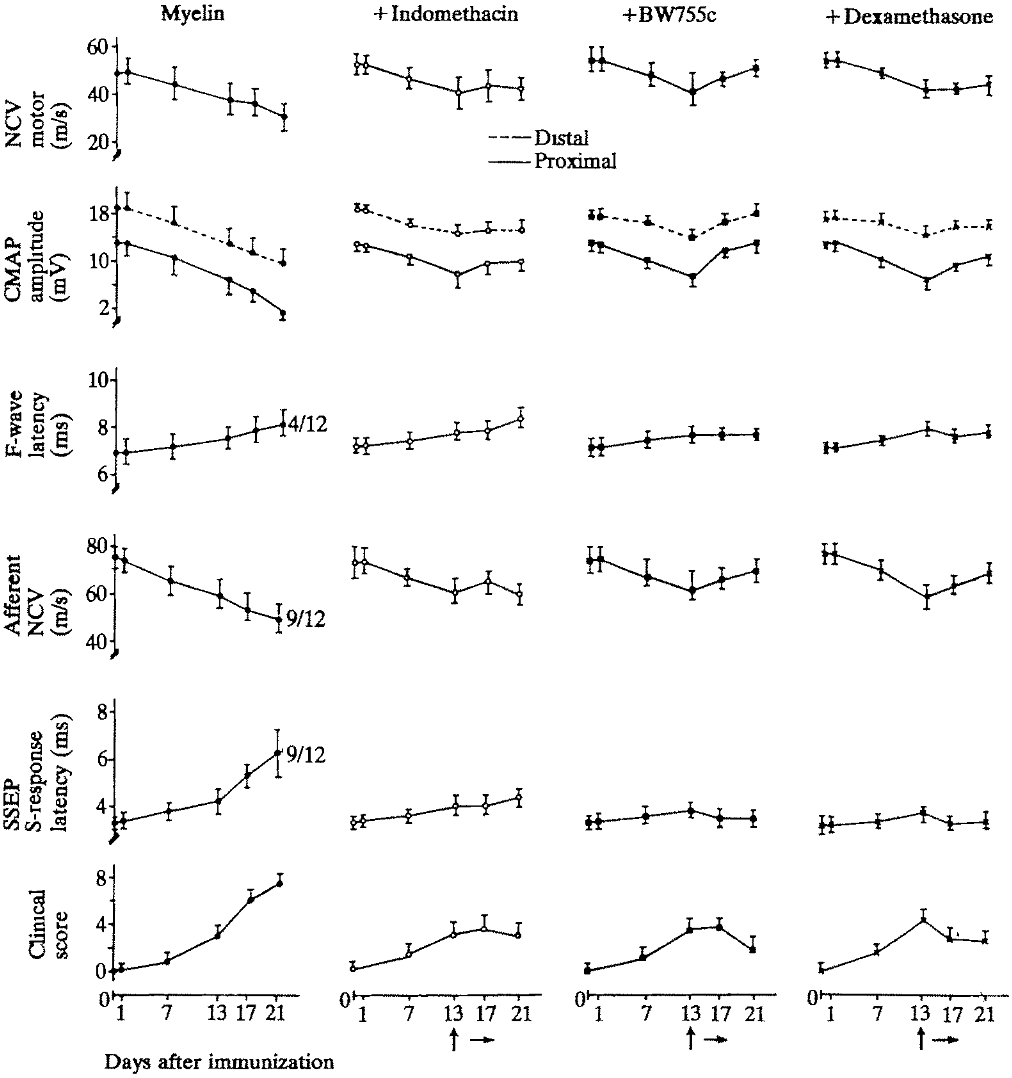


FIG. 7 Time course of electrophysiological findings in rats receiving treatment after the appearance of clinical signs (from day 13 p.i.). For explanation, *see* legend to fig. 3.

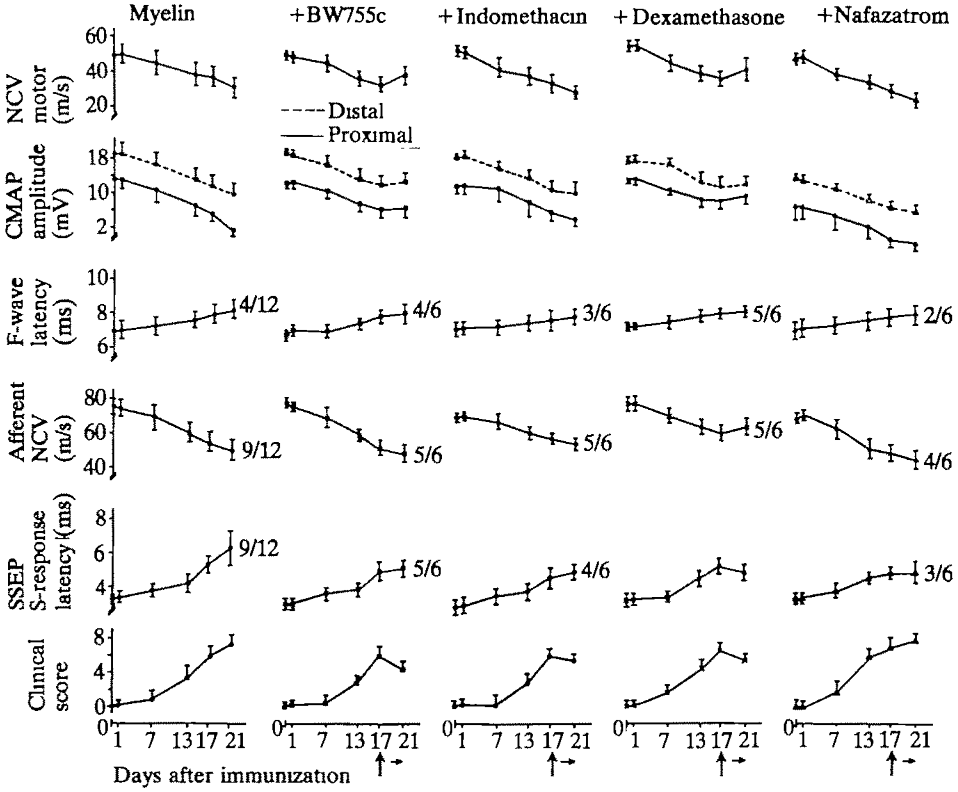


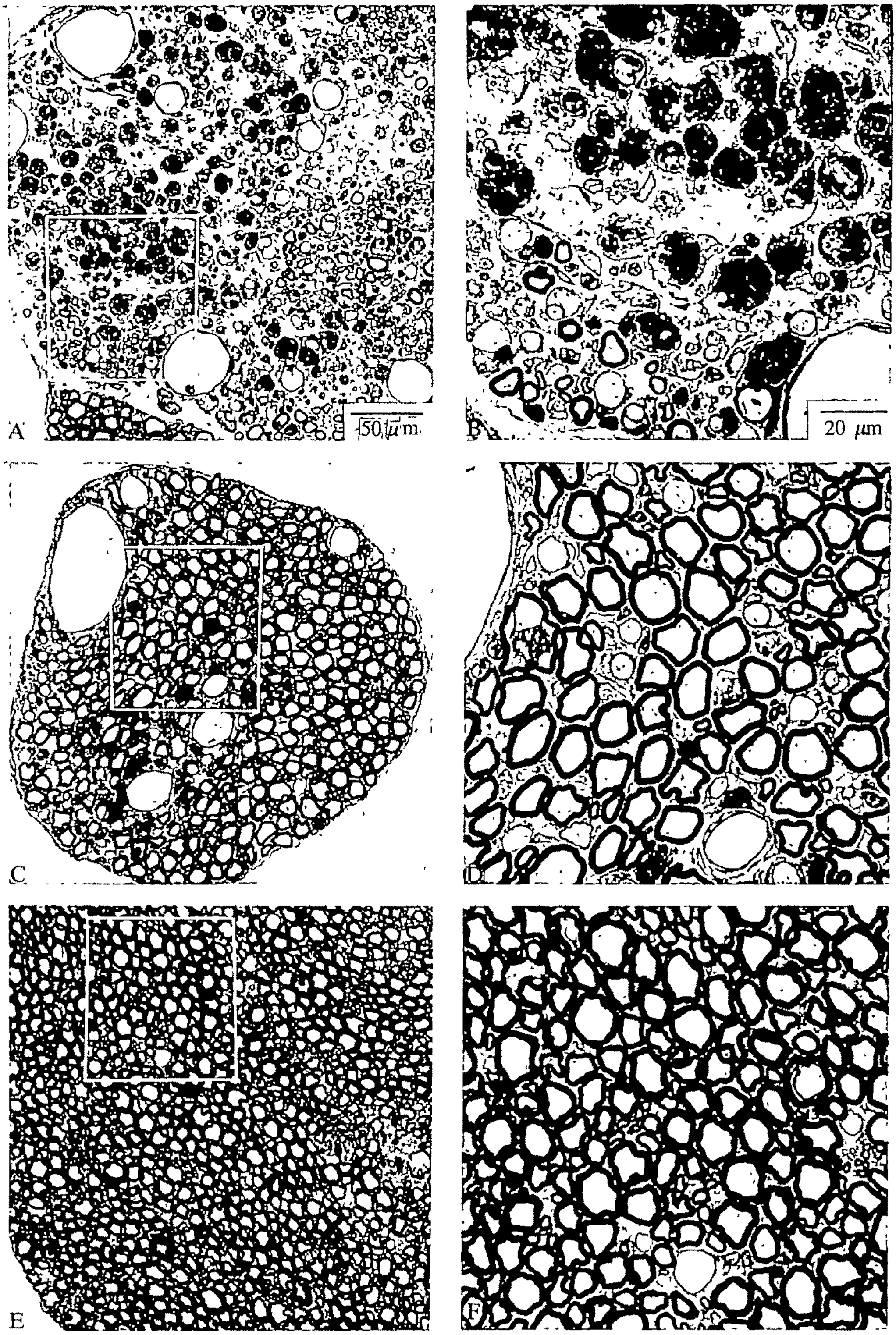
FIG. 8. Time course of electrophysiological findings in rats receiving treatment late in the course of the disease (from day 17 p.i.) For explanation, *see* legend to fig 3. Numbers indicate proportion of animals in which an F wave, an S response of the lumbar SEP or a mixed afferent potential was elicitable.

Corticosteroid treatment

High dose dexamethasone prevented the clinical and electrophysiological signs of EAN when administration was initiated on day 7 p.i. (figs 3, 4, 6). When steroid application was started after the appearance of the first signs of EAN on day 13, clinical scores and electrophysiological findings reverted almost to pretreatment values (fig. 7). Even when commencement of treatment was delayed until day 17, animals were affected to a lesser degree than sham-treated controls at the endpoint of the study (fig. 8).

Effects of cyclo-oxygenase and lipoxygenase blockers

Oral administration of both indomethacin and BW755c notably changed the course of the disease. Indomethacin when given from days 7 or 13 onwards suppressed the development of clinical signs or partially reverted them. Electrophysiologically, slight changes occurred (figs 3, 4, 6, 7). Motor NCV, for example, decreased by about 25% and F wave latencies were prolonged, but no failures in obtaining F waves occurred. The configuration of the S response of the lumbar SEP


A
50 μm
B
20 μm
C
D
E
F

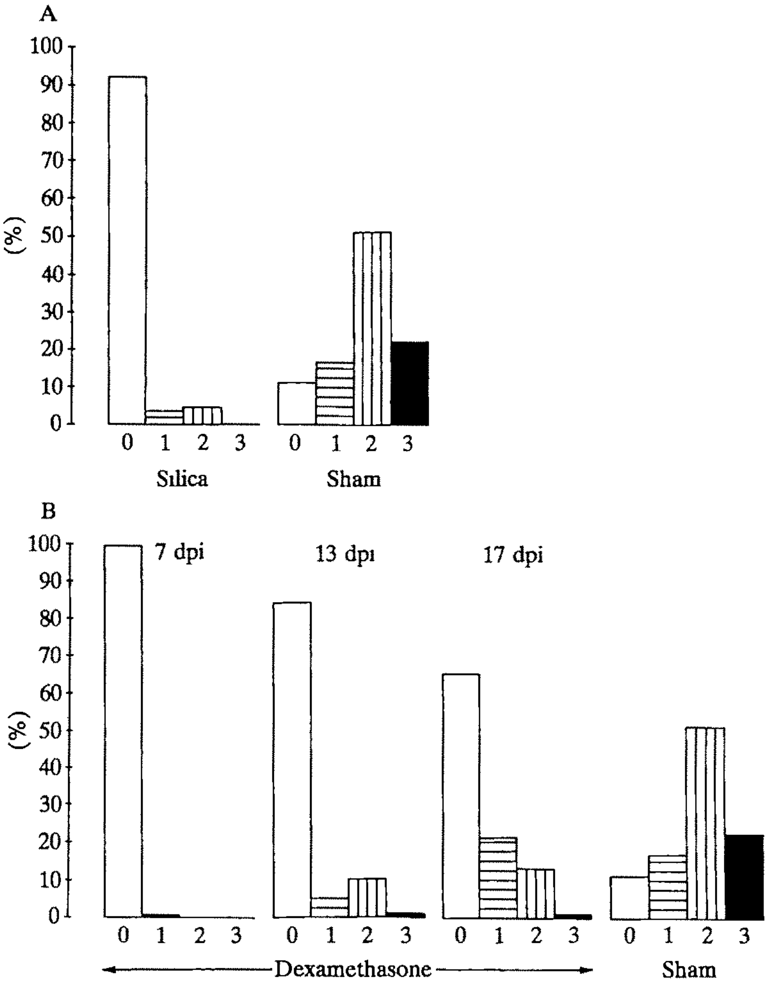


FIG. 10 Semiquantitative analysis of histological changes. Comparison of sham, silica (A) and dexamethasone-treated (B) rats. The columns indicate the percentage of intraneural perivascular areas that were assigned to one of the four grades on the semiquantitative score (*see* Methods), 250 such perivascular areas were examined per animal. Data are accumulated for each treatment group.

was largely preserved with a mild delay (figs 3, 6, 7). Rats that had received BW755c were clinically and electrophysiologically not significantly changed when compared at the beginning and termination of the experiments, independent of whether treatment was started on days 7 or 13 p.i. (figs 3–8). In contrast, the lipoxygenase inhibitor

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FIG 9. Representative micrographs of 1 μm Epon sections at the S1 root level from animals that had received sham treatment (A, B), or treatment with BW755c (C, D), or dexamethasone (E, F) after onset of clinical signs on day 13 p i Note extensive cellular infiltration and demyelination both in the immediate vicinity of as well as at a distance from intraneural vessels in A and B There are only occasional infiltrates and a few demyelinated fibres in the BW755c-treated group, whereas sections from the dexamethasone-treated group show almost no significant pathological alterations B, D and F (all at same magnification) are higher magnifications taken from A, C and E, respectively (frame), the latter again all at the same magnification

nafazatrom had little effect on the course and outcome of the disease (data not shown).

Morphological evaluation of treatment results

In untreated EAN animals the predominant pathological alterations observed on day 21 were cellular infiltration and demyelination, the latter occurring both in proximity to and distant from blood vessels (figs 1, 9, 10). Treatment with the various compounds except for nafazatrom markedly suppressed EAN which was in close correlation with clinical and electrophysiological findings in each group (figs 9–11).

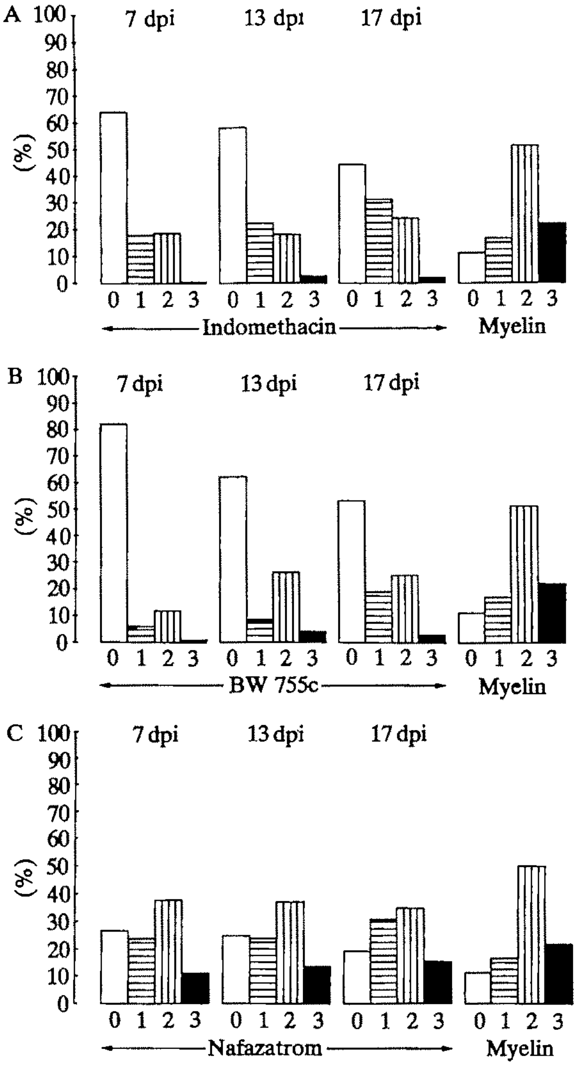

FIG 11. Semiquantitative analysis of morphological alterations in indomethacin (A), BW755c (B) and nafazatrom-treated (C) rats. For explanation, *see* legend to fig. 10.

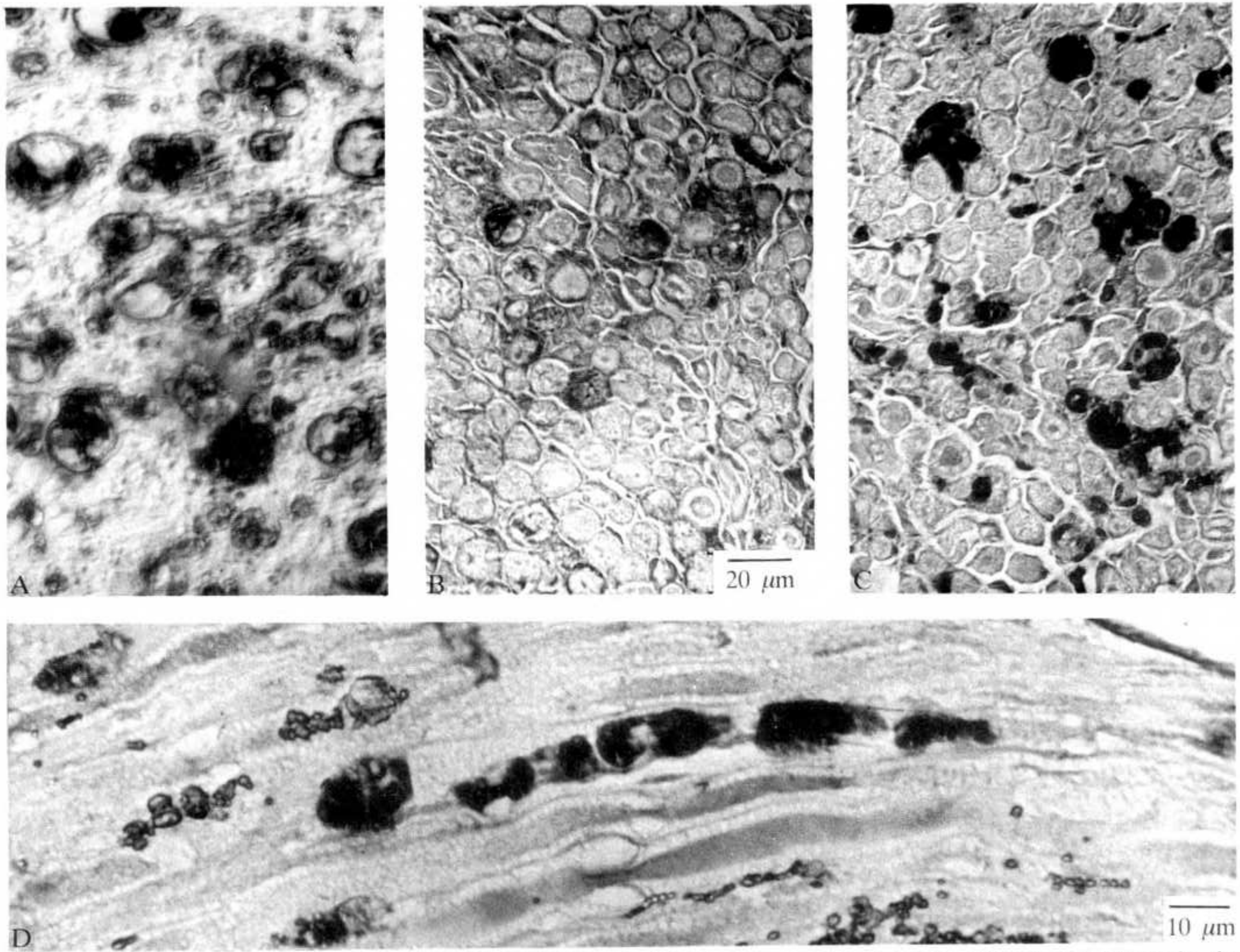

FIG. 12. Immunocytochemical studies. S1 root sections were examined for the presence of ED1-positive cells, i.e. macrophages, by the avidin-biotin-complex method. Shown are representative examples. A, sham-treated controls. B, treatment with BW755c from day 13 p.i. C, treatment with BW755c from day 17 p.i. D, longitudinal section of an S1 root from an animal that had received BW755c from day 13 p.i. Note heavy infiltration of roots by macrophages in the sham-treated group and almost complete absence of these cells in the early treatment group. All at same magnification.

Further, comparison between preventive administration (started on day 7) and therapeutic intervention (starting on day 13 or 17) reveals that, as might be expected from clinical observation, tissue damage was more severe in the latter group as indicated by a higher proportion of grades 1 and 2 abnormalities (figs 10, 11). Again, this correlated closely with the neurographic findings. On semiquantitative histological analysis dexamethasone proved to be the most efficacious suppressive agent. Finally, no relapses were noted within 24 days of discontinuing treatment with any of the effective agents.

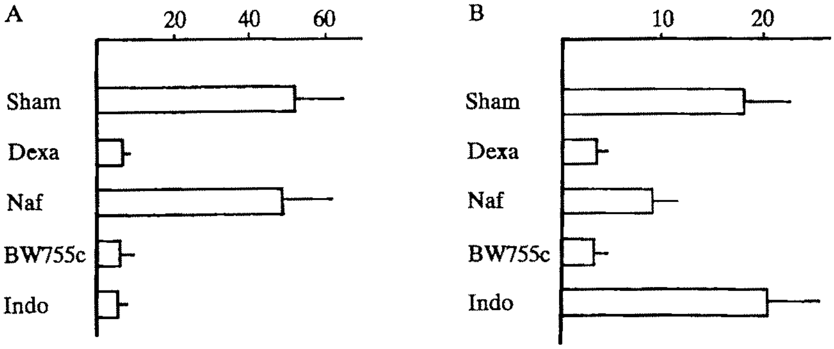

FIG. 13. Ex vivo generation of prostaglandin E (A) and leukotriene C_4 (B) by macrophages from sham-treated and treated rats (Dexa = dexamethasone; Naf = nafazatrom, Indo = indomethacin). Adherent peritoneal macrophages from rats of the various treatment groups were challenged with 40 μg/ml zymosan and kept in short-term culture. After 6 h, supernatants were collected and later radioimmunoassayed for prostaglandin E and leukotriene C_4 content Drugs known to act on the cyclo-oxygenase pathway markedly suppressed prostaglandin E release. Joint inhibitors of cyclo-oxygenase and lipoxygenase (dexamethasone and BW755c) both reduced PGE and LTC_4 formation. The selective lipoxygenase blocker nafazatrom did not affect PGE production. It was only partially effective in suppressing leukotriene generation. Results are expressed per mg of protein and respresent means of triplicate cultures.

Immunocytochemical examination revealed that the cellular infiltrates in the lesions from sham-treated animals were almost completely comprised of macrophages which were identified by the monoclonal antibody ED1 (fig. 12). Treatment of EAN rats with silica, dexamethasone, BW755c or indomethacin dramatically reduced the number of detectable ED1-positive cells in all root and nerve sections (fig. 12).

Effects of drug treatment on eicosanoid generation ex vivo by cultured macrophages

When the synthesis of arachidonic acid metabolites by macrophages ex vivo was compared in animals from the different treatment groups it was noted that in vivo administration of dexamethasone and BW755c markedly diminished PGE and LTC_4 production, while indomethacin and nafazatrom selectively reduced PGE or LTC_4 generation, respectively (fig. 13).

DISCUSSION

This correlative study of clinical, electrophysiological and morphological manifestations in EAN was undertaken to examine in more detail the functional importance of macrophages and to address in particular the role of eicosanoids in the pathogenesis of this inflammatory demyelinating polyneuropathy. To this end, pharmacological compounds that affect pertinent macrophage effector functions were administered, and their influence on the course of EAN was studied. Macrophages can mobilize arachidonic acid from membrane phospholipids through the action of the enzyme phospholipase A_2 and metabolize this 20-carbon unsaturated fatty acid either by the cyclo-oxygenase pathway to prostaglandin, the most important derivative being prostaglandin E (PGE), and thromboxane or by the lipoxygenase pathway to 5-hydroxy-eicosa-6,8,11,14-tetraenoic acid (5-HETE) and the leukotrienes of which leukotriene C_4 (LTC_4) is the predominant product (fig. 2) (Humes *et al.*, 1977; Rouzer *et al.*, 1980; Bonney and Humes, 1984; Needleman *et al.*, 1986).

Prostanoids and leukotrienes have been shown to exhibit multiple proinflammatory properties. PGE and prostacyclin cause vasodilatation and increased blood flow, contribute to oedema formation by augmenting vascular permeability, and thus facilitate influx of inflammatory cells into and their accumulation at inflamed regions (reviewed by Morley, 1981; Williams, 1983; Davies *et al.*, 1984). Leukotrienes also enhance vasopermeability, act as chemoattractants for leucocytes, and cause macrophages to release toxic oxygen species (Hartung, 1983; Lewis and Austen, 1984; Samuelsson *et al.*, 1987). Among leucocytes, macrophages are by far the predominant source of eicosanoids, and it is through the release of these highly active substances that these cells mediate at least a substantial part of their effects in an inflammatory reaction (Gemsa, 1981; Morley, 1981; Adams and Hamilton, 1984).

In confirmation of the report by Tansey and Brosnan (1982) and the more detailed morphological investigation of Craggs *et al.* (1984), macrophage blockade by repetitive intraperitoneal injections of silica quartz dust was very efficacious in suppressing the disease. Since silica (1) is a rather crude and global inhibitor of macrophages, (2) may exert additional effects on other immunocompetent cells (Levy and Wheelock, 1975; Kagan and Hartmann, 1984), and (3) obviously has no prospect of gaining therapeutic value in the human counterpart of EAN, we evaluated more specific inhibitors, focusing on agents that are known to interfere with arachidonic acid metabolism: dexamethasone, indomethacin, BW755c and nafazatrom (Higgs and Vane, 1983; Bonney and Humes, 1984).

The effects of corticosteroids on the course of EAN have been studied previously, although neurographic monitoring has not been reported (Abramsky *et al.*, 1977; Hughes *et al.*, 1981; King *et al.*, 1985). Results regarding efficacy of corticosteroid treatment have been conflicting. When rabbits and guinea pigs immunized with P_2 protein received alternate day injections of 20–50 mg hydrocortisone (equivalent to 1–2.5 mg dexamethasone) for a period of 1 month, starting on day 2 p.i., Abramsky *et* *al.* (1977) found a complete prevention of clinical manifestation of EAN with only minor histopathological damage in 15% of the guinea pigs. In myelin-induced EAN of Lewis rats, prednisone (10 mg/kg body weight) when administered during the phase of overt neurological signs was observed to hasten clinical improvement and to reduce peak severity of the disease (Hughes *et al.*, 1981). King *et al.* (1985), studying steroid effects on EAN in Lewis rats, confirmed the earlier findings of Hughes *et al.* (1981), although the effects achieved were not as marked. We obtained complete prevention and considerable therapeutic effects by applying dexamethasone twice daily in high doses. Although steroid-treated animals lost weight (mean 16 ± 5% of initial weight vs 29 ± 7% in sham-treated controls), probably due to the catabolic action of the glucocorticoids, no other serious side-effects were encountered during the course of the experiment or on histological examination. The more marked effects that we have noted in our present study are most probably explained by the higher dose of corticoids that we administered (4 mg of dexamethasone being roughly equivalent to 20 mg prednisolone (Haynes and Murad, 1985), twice the dose employed by King *et al.*, 1985). Further, our dosing schedule differed in that we administered injections twice daily, a regimen that is likely to result in more sustained tissue levels of the drug.

Of the 3 compounds that exert their pharmacological actions exclusively by interference with arachidonic acid conversion, indomethacin and BW755c proved to be potent agents in preventing EAN and were effective also when given at the onset of clinical signs or even late in the course of the disease. Morphologically, there was a conspicuous absence of cellular infiltrates at roots as well as at more distal parts of the sciatic nerves in animals that had received prophylactic treatment with either compound. Indomethacin, BW755c and dexamethasone, have been demonstrated to prevent immigration of phagocytic leucocytes to inflamed tissues in experimental inflammatory models such as carageenin-induced pleurisy or rat paw oedema (Higgs *et al.*, 1980; Higgs, 1984) at dosages comparable to those employed here. To assess whether this mode of action is operative in the early amplification phase of the effector stage in EAN we had to employ a method that would identify infiltrating cells as macrophages. Previous immunocytochemical studies on the cellular composition of the nerve infiltrates in EAN, using monoclonal antibodies of the OX series and W3/13 and W3/25, have revealed that at the height of the disease Ia-positive cells are abundant (Olsson *et al.*, 1984; Ota *et al.*, 1987). Since MHC class II antigens are not exclusively expressed on the surface of macrophages, the unequivocal identification of the stained cells as macrophages has not been forthcoming. With a monoclonal antibody specifically recognizing rat macrophages this has now become feasible (Dijkstra *et al.*, 1985). We observed large numbers of ED1-positive cells in the inflammatory lesions.

Administration at an early phase of the disease of the above-mentioned inhibitors of arachidonate conversion resulted in a marked reduction of ED1-stained cells. However, when drug administration was instituted after appearance of overt neurological signs and electrophysiological evidence of nerve dysfunction cellular infiltrates were present in the nerve although to a much lesser degree than found in sham-treated controls. The beneficial effects of the agents at this stage can therefore not solely be attributed to blocking macrophage influx into the lesion. It is likely that those macrophages present in the nerve can no longer generate arachidonic acid-derived chemotactic signals, and hence further accumulation of cells with ensuing amplification of inflammatory damage is prevented. Nafazatrom, selectively blocking 5-lipoxygenase activity (Bonney and Humes, 1984), was only slightly effective in suppressing EAN.

While steroids have a range of immunosuppressive effects particularly related to T lymphocyte functions and the afferent limb of the immune response (Snyder and Unanue, 1982; Kelso and Munck, 1984; Bach and Strom, 1985; Goodwin *et al.*, 1986; Reed *et al.*, 1986), most of their anti-inflammatory actions in the effector phase of an immune response can be attributed to interference with arachidonic acid metabolism. Glucocorticoids induce a protein (lipocortin/lipomodulin/macrocortin) that blocks phospholipase A_2 (see fig. 2). Consequently, the supply of substrate for the conversion to prostanoids and leukotrienes is curtailed (Flower, 1986). However, steroids could also work by diminishing macrophage secretion of enzymes (Werb, 1978) which have been implicated in the pathogenesis of EAN (Trotter and Smith, 1986). Indomethacin blocks cyclo-oxygenase and hence prevents formation of prostaglandins and thromboxane (Higgs and Vane, 1983). Whether it can also inhibit phospholipase A_2 is controversial. BW755c is a potent joint inhibitor of cyclo-oxygenase and lipoxygenase activity (Higgs and Vane, 1983).

In the experiments reported here we found that inhibition of prostaglandin synthesis was crucial for the prevention and suppression of EAN. In contrast, the efficacy of indomethacin and the poor effects of the specific lipoxygenase blocker nafazatrom would suggest that leukotrienes do not play a major role on their own. It is likely that nafazatrom does not completely inhibit leukotriene formation as shown in our ex vivo experiments. Therefore, some contribution of lipoxygenase-derived arachidonic acid metabolites cannot yet be excluded. In particular, even low levels of leukotrienes may act in a complementary way along with prostanoids to produce a maximal inflammatory response (Williams, 1983; Higgs, 1984). The ex vivo experiments clearly demonstrate that in vivo blockade of arachidonate metabolism does in fact reduce macrophage release of eicosanoids. Apart from T cells which generate only negligible amounts of arachidonate conversion products, macrophages are the main cellular component of the infiltrate in nerve, and with progression of the disease they evolve as the predominant cell type (Waksman and Adams, 1955; Lampert, 1969; Raine, 1985). Thus they are the relevant target for the drugs employed. We consider a contribution of neutrophils unlikely because they generally are present only in the early phase of an inflammatory response and synthesize significantly lesser amounts of eicosanoids than macrophages (Gemsa, 1981).

Finally, apart from investigating the pathogenic role of macrophage-derived eicosanoids we have extended previous neurophysiological studies on EAN which had demonstrated the typical electrophysiological features of a demyelinating neuropathy, namely conduction slowing, failure and block. Cragg and Thomas (1964) performed studies in vitro on excized sciatic nerves from guinea pigs. Hall (1967) recorded in vivo from surgically exposed dorsal and ventral roots in the same model, and Kaeser and Lambert (1962) briefly mention results obtained in living guinea pigs with both EAN and EAE. Prolonged F wave conduction time in a small percentage of guinea pigs and rabbits with EAN was noted by Tuck *et al.* (1982). However, there is only one previous report presenting serial studies of the electrophysiological changes developing in actively induced EAN (Rostami *et al.*, 1984). In that study, evolving abnormalities of motor nerve conduction such as failure, block, increased F response latencies, were noted to occur in a temporal sequence similar to our observations. We have extended these observations by recording mixed afferent potentials and lumbar SEPs. We found that commencing on day 7 p.i. latencies of mixed afferent evoked potentials became increasingly delayed accompanied by reductions in amplitude. These findings reflect the involvement of sensory fibres in EAN (Hall, 1967). Since pathological damage in EAN occurs with highest frequency and maximum severity at the most proximal segments of the sciatic nerves (Waksman and Adams, 1955; Smith *et al.*, 1979; Raine, 1985) we also recorded lumbar evoked SEPs. Our studies on lumbar SEPs in myelin-induced EAN demonstrate the same abnormalities, that is, delayed latencies, and a reduction in amplitude of the S response and its temporal dispersion, as those that we have described before in the adoptive transfer model of the disease (Heininger *et al.*, 1986).

In summary, from the proven efficacy of pharmacological blockers of arachidonic acid conversion to suppress the disease we can conclude that macrophage-derived eicosanoids are instrumental in imparting damage to the nerves in EAN and are thus involved in the pathogenesis of this acute inflammatory demyelinating disorder. The possible role of macrophages in the initiation of immunoinflammatory responses (Unanue and Allen, 1987) culminating in peripheral nervous system damage is yet to be investigated. In view of the fact that so far the only therapeutic modality that has been convincingly proven to be of value in the acute Guillain-Barré syndrome is plasma exchange (Guillain-Barré Syndrome Study Group, 1985), our results in the animal model may guide future trials designed to evaluate drug treatment that may provide benefit in the early effector phase of this immunologically mediated disorder.

ACKNOWLEDGEMENTS

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VERBAL MEMORY AND LEARNING IN UNILATERAL POSTERIOR CEREBRAL INFARCTION

A REPORT ON 30 CASES

by D. Y. VON CRAMON, N. HEBEL *and* U. SCHURI

(*From the Neuropsychological Department, München-Bogenhausen City Hospital, München, FRG*)

SUMMARY

Unilateral posterior cerebral infarction, sparing memory-related structures in the diencephalon, may represent a means of investigating the role of hippocampal afferents and the parahippocampal areas in memory processing. Among 30 patients with unilateral posterior cerebral infarction a group of 12 subjects with left-sided lesions suffered from marked *verbal* memory and learning dysfunction, whereas the remaining subjects with left or right-sided lesions showed no obvious memory deficit. The impairment was most prominent for verbal learning tasks, while recall of isolated and complex verbal information appeared to be less affected. In some cases the memory disturbance could be detected up to one year after the causative event.

Analysis by CT scanning revealed a coincidence of mnestic disturbances with lesions around the left collateral sulcus affecting the posterior parahippocampal gyrus and the collateral isthmus. The latter term refers to the fibre stem of the medial temporal lobe limited by the floor of the lateral ventricle and the depth of the collateral sulcus. Through this bottle-neck, bidirectional fibres run between the posterior parahippocampal gyrus and different sensory-specific and multimodal association areas. A lesion within the collateral isthmus or, more particularly, a combined lesion of the collateral isthmus and the posterior parahippocampal gyrus, deprives the hippocampus itself of its main afferent projection source and, on the other hand, prevents dissemination of the hippocampal output to widespread neocortical areas.

It cannot be excluded, at least in some cases, that small lesions in the fimbria–fornix route, in the retrosplenial cortex or in the hippocampal formation itself also contribute to the memory and learning dysfunction. However, these small lesions, so far as they could be detected by CT scanning, were present in patients with and without memory disturbances.

INTRODUCTION

Mnestic disturbances are a well-known clinical feature following bilateral temporomesial damage due to vascular lesions. Although early reports on this topic date back to the turn of the century, case reports with detailed data on both memory performance and involved anatomical structures are rare. There is a great deal of

Correspondence to: Professor D Y von Cramon, Munchen-Bogenhausen City Hospital, Englschalkingerstrasse 77, D-8000 München 81, FRG.

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evidence that bilateral (homologous) posterior cerebral infarctions (PCI) cause persistent amnesia. It is still controversial, however, whether unilateral PCI may also yield lasting mnestic disturbances (*see* Benson *et al.*, 1974).

As far as we are aware, only 3 pathoanatomical studies have demonstrated that a left-sided PCI has been followed by severe memory dysfunction (*see* Geschwind and Fusillo, 1966; Mohr *et al.*, 1971; Caplan and Hedley-Whyte, 1974). Although not fully comparable with respect to formal test procedures, poor verbal memory performance was observed in all 3 cases. The case report of Caplan and Hedley-Whyte (1974) documented an example of a severe memory disturbance lasting 6 months related to a unilateral PCI; after that time memory functions were reported to have greatly improved.

Recent clinical studies (without pathoanatomical evidence) also suggest the occurrence of memory and learning disturbances after unilateral PCI. Benson *et al.* (1974) reported 4 cases with deficits in verbal learning tasks after left-sided PCI. Fisher (1986) observed unspecified memory disturbances in 24 patients with PCI of the dominant hemisphere. On the other hand, in 2 cases with extensive left-sided PCI, no obvious memory disturbances were reported by Marti-Masso *et al.* (1981). In neither study, however, did the authors provide detailed information on memory testing and localization of the PCI.

As to the anatomical considerations, interest has hitherto focused on the hippocampal formation (*see* Benson *et al.*, 1974; Woods *et al.*, 1982; Markowitsch and Pritzel, 1985), whereas adjacent structures, such as the parahippocampal gyrus with its afferent and efferent fibre projections, received only minor attention. It was the aim of the present study to extend the hypotheses concerning the anatomical structures that may be relevant with respect to verbal memory deficits following unilateral PCI. In order to avoid unreliable results when testing memory function in patients with all kinds of visual and reading dysfunction, we restricted the investigation to the study of verbal memory function with auditory presentation of the stimulus material.

NEUROANATOMICAL CONSIDERATIONS

Memory-related temporomesial brain structures supplied by the posterior cerebral artery (PCA)

The PCA supplies large parts of the retrocommissural hippocampus by one to four hippocampal branches which usually arise shortly after the confluence with the posterior communicating artery. They enter the hippocampal sulcus and follow it in an anterior direction approximately parallel to the hippocampus. In 50% of their cases Zeal and Rhoton (1978) found only a single hippocampal branch (fig. 1).

The secondary hippocampal branches travel to the dentate gyrus and the subiculum; some branches continue in the direction of the hippocampal sulcus; perforating branches terminate in a polyanastomotic network within the granular layer. In some cases a single vessel spreads out to the uncus hippocampi. The fimbria

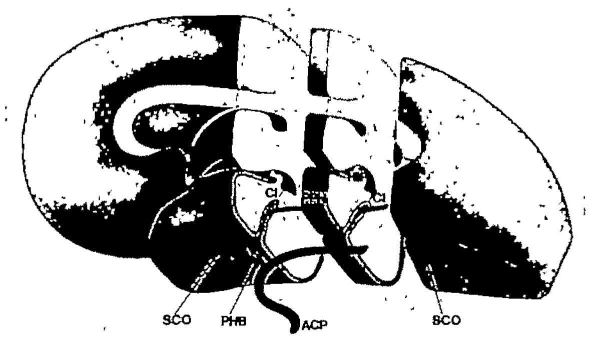


FIG. 1. Mediobasal view of right cerebral hemisphere (schematic drawing). The major part of the fibre tracts from and to the posterior parahippocampal (PPH) gyrus have to pass the anatomical narrowing between the lateral ventricle and the collateral sulcus (collateral isthmus, CI) This white matter stem is supplied by parahippocampal branches (PHB) of the PCA running in the depth of the collateral sulcus (SCO) Infarction within the supply territory of these branches can lead to functional disconnection of posterior hippocampal formation and posterior parahippocampal gyrus

hippocampi (including its taenia) and probably the paraventricular part of the pes hippocampi belong to the territory of the anterior choroidal artery (Hens and Van den Bergh, 1977), the crura fornicis being supplied by a lateral branch of the posterior choroidal artery.

The posterior two-thirds of the parahippocampal gyrus are supplied by branches of the PCA which fan out from the temporo-occipital artery in the ambiens cistern. These branches supply the anterior part of the territory of the temporo-occipital artery. In about 40% of subjects (Zeal and Rhoton, 1978) there is a separate middle temporal artery giving rise to the parahippocampal branches. These branches traverse the parahippocampal gyrus running laterally into the collateral sulcus (Lecaque *et al.*, 1978). There are always some branches following the depth of this sulcus; more laterally they reach the lateral occipitotemporal gyrus. Parahippocampal branches also supply the white matter of the fourth and fifth temporal convolution up to the floor of the lateral ventricle.

Most of the bidirectional fibre tracts connecting the posterior parahippocampal gyrus (PPH) with different cortical association areas (*see* Van Hoesen, 1982) have to pass the territory of these penetrating white matter branches of the PCA through a bottle-neck between the bottom of the collateral sulcus and the floor of the lateral ventricle (fig. 1). This bottle-neck will be referred to as the collateral isthmus (CI). The region around the collateral sulcus constitutes the 'gravity' centre of infarction within the temporal territory (Kleihues and Hizawa, 1966). This implies that the CI, which contains the major part of the temporomesial projections, is localized in the critical zone of the supply territory. The anterior third of the parahippocampal gyrus lies within the territory of the anterior choroidal artery (*see* Yasargil *et al.*, 1985).

At its terminal distribution, the PCA supplies the retrosplenial cortex (or isthmus gyri cinguli) or the underlying cingular bundle by a branch of the parieto-occipital artery.

CASE MATERIAL

All patients with a diagnosis of unilateral posterior cerebral infarction admitted consecutively during 1985 and 1986 to our neuropsychological department were included in the study. Patients having additional lesions outside the ipsilateral territory of the PCA and patients with additional lesions in the polar/paramedian thalamic or posterior choroidal territory were excluded. We tested 30 patients (14 females, 16 males) with unilateral PCI (identified according to CT radiographic criteria; *see* below); 20 patients had selective left-sided and 10 right-sided lesions. Their ages ranged from 25 to 73 yrs with a median of 59 yrs; there was no essential difference in the age distribution between left- and right-sided PCI. The time from the occurrence of the lesion ranged from 2 to 12 months (median 4 months) in patients with left-sided PCI and 2 to 24 months (median 5 months) for right-sided PCI. All patients were native German speakers and all were right-handed except for 2 cases (10 and 13) who were ambidextrous.

The main clinical data are given in Table 1.

NEUROPSYCHOLOGICAL TESTING

In 19 patients there was a complete homonymous hemianopia with sparing of 1.5 deg visual angle in the central field. An inferior quadrantanopia occurred in only 1 patient. In 7 cases the size of the residual visual field ranged between 4 and 16 deg visual angle on the horizontal axis.

Visual search was severely impaired in the majority of cases, partly because of the visual field defect and partly due to an additional disorder of visual exploration. Simple visual reaction times were normal in all patients; visual choice reaction times were slightly impaired in 4 cases. In 8 cases (left and right PCI) reading was hampered by hemianopic dyslexia. In another 8 cases, all with left-sided PCI and severe memory dysfunction, symptoms of pure alexia were superimposed on hemianopic reading errors.

In all cases pure tone thresholds at standard audiometric frequencies were within normal limits, as were simple acoustic reaction times and acoustic choice reaction times. The examination of acoustic information processing with the PASAT (Gronwall and Wrightson, 1974) yielded average results, and those for the Token Test (German form, Orgass, 1976) were normal in all patients. Formal analysis of spontaneous speech with the AAT (Aachener Aphasie Test, Huber *et al.*, 1982) did not reveal any phonological or syntactical errors.

Although 9 out of 12 patients with memory and learning deficits complained of impairment in expressive language abilities, word-finding difficulties were not easily detected in spontaneous speech and did not emerge in various naming tasks.

Sensorimotor deficits were present in about half of the patients. Hemiparesis was mild or minimal in all cases. Somatosensory deficits in all modalities (including various kinds of sensory distortions) could either be attributed to an additional thalamic lesion in the territory of the thalamogeniculate arteries (Cases 4, 6, 8, 10, 11, 19, 20, 26, 27) or to brainstem lesions in the territory of the peduncular branches of the PCA (Cases 1, 5, 23, 24, 25, 30).

General intelligence as tested with the German short version of the Wechsler Adult Intelligence Scale (Dahl, 1972) was normal in all patients. There was, however, a significantly impaired performance in the 'information' subtest for those patients with objective memory and learning deficits. No such difference could be found for the subtest 'similarities'.

Examination of memory and learning

All verbal learning and memory tasks were administered exclusively acoustically (for reasons, *see* above). The span of immediate verbal recall was tested by the digit span as well as by free recall of a 10-word list. The nouns with at least a 6.0 concrete rating on Paivio's scale (Paivio *et al.*, 1968) were spoken at a rate of 1 noun/s. Memory for complex verbal information was tested by free recall of a 57-unit story immediately following auditory presentation as well as 48 h later.

TABLE 1 MAIN CLINICAL DATA

Case no.	*Age/sex (yrs)*	*TSL (m)*	*IT*	*Side*	*MEM*	*VD*	*LD*
Group 1							
1	73 M	4	STO	L	+	CH, VSI	WFD
2	61 M	5	T	L	+	CH, VSI	PA
3	52 F	11	T	L	+	CH, VSI	WFD
4	65 M	7	MB	L	+	8	WFD, PA
5	61 M	2	MB	L	+	CH, VSI	WFD, PA
6	60 M	11	T	L	+	CH, VSI	WFD, PA
7	58 F	7	MB	L	+	CH, VSI	WFD
8	64 F	4	T	L	+	CH, VSI	PA
9	49 M	12	T	L	+	10, VSI	WFD, PA
10	51 M	2	STO	L	+	CH, VSI	WFD, PA
11	30 F	3	T	L	+	CH	WFD
12	55 F	3	MB	L	+	CH, VSI	PA
Group 2							
13	42 M	12	MB	L	–	15	
14	62 F	4	AP	L	–	CH, VSI	
15	69 M	4	C	L	–	CH, VSI, HD	
16	45 F	2	C	L	–	10	
17	69 M	6	MB	L	–	4, HD	
18	55 M	2	MB	L	–	IQ, HD	
19	44 F	3	C	L	–	5, HD	
20	25 F	2	AP	L	–	4	
Group 3							
21	45 M	13	T	R	–		
22	70 F	2	MB	R	–	10	
23	69 F	10	MB	R	–	CH, VSI	
24	45 M	24	T	R	–	CH, VSI	
25	49 M	5	STO	R	–	CH, VSI, HD	
26	65 F	10	MB	R	–	CH, VSI, HD	
27	65 F	9	MB	R	–	CH, VSI	
28	59 M	3	T	R	–	CH, VSI, HD	
29	56 M	4	MB	R	–	CH, VSI	
30	64 F	5	MB	R	–	5, HD	

TSL = time since lesion (in mos); IT = infarction type, STO = subtotal PCI (all 3 cortical branches); MB = mediobasal PCI (temporal and calcarine arteries), AP = apical PCI (calcarine and parieto-occipital arteries); T = temporal artery infarction; C = calcarine artery infarction; MEM = severe memory deficit; VD = visual perceptual deficit (CH = complete homonymous hemianopia, IQ = inferior quadranopia; in all cases with incomplete homonymous hemianopia the residual central field on the horizontal axis is indicated in degrees; VSI = visual search impairment; HD = hemianopic dyslexia); LD = linguistic disorder (WFD = word finding difficulties, PA = pure alexia).

Verbal learning was studied with a word paired-associates learning task and with Rey's test of auditory verbal learning (Rey, 1964). The material of the paired-associates learning task consisted of 8 distant pairs of nouns (concreteness) 6.0 on Paivio's scale; *see* above); they were read to the patients at intervals of 3 s; free recall was tested immediately after each of 4 learning trials.

Autobiographical memory was assessed in a semistructured interview. Subjective memory was studied with a German version of the subjective memory questionnaire (Bennett-Levy and Powell, 1980), as well as from free reports of memory problems in everyday life.

NEUROPSYCHOLOGICAL RESULTS

The results of the examination of memory and learning are summarized in Tables 2 and 3.

Cases 1–12 with left-sided PCI (group 1) were all impaired in both tests of verbal learning. In a small fraction of cases additional problems occurred in recall of the 10-word list (Cases 5–7) or of the 57-unit story (Cases 1–4). For recall of the word list, a separation of a short-term and long-term components was made using the estimate of Tulving and Colotla (1970). According to their criteria, the respective number of recalled items from short-term and long-term storage of Cases 5–7 were 2/2, 0/3 and 1/3, respectively.

Remote memory deficits occurred only in group 1; 9 patients had difficulties in retrieving previously well-known names of persons, places, etc. Two patients showed a substantial loss of professional knowledge.

Comparison of subjective memory (based on spontaneous reports as well as on items from the subjective memory questionnaire) with objective test data revealed

TABLE 2 ASSESSMENT OF MEMORY AND LEARNING (INDIVIDUAL PERFORMANCE)

Case no.	*Side*	*DS*	*WL*	*ST1*	*ST2*	*PAL*	*AVL*	*RM*
Group 1								
1	L	+	+	−	−	−	−	−
2	L	+	+	−	−	−	−	−
3	L	+	+	−	−	−	−	−
4	L	+	+	+	−	−	−	−
5	L	+	−	+	+	−	−	−
6	L	+	−	+	+	−	−	+
7	L	+	−	+	+	−	−	+
8	L	+	+	+	+	−	−	−
9	L	+	+	+	+	−	−	−
10	L	+	+	+	+	−	−	−
11	L	+	+	+	+	−	−	−
12	L	+	+	+	+	−	−	+
Group 2								
13–20	L	+	+	+	+	+	+	+
Group 3								
21–30	R	+	+	+	+	+	+	+

L, R = PCI left/right; DS = digit span; WL = free recall of a word list; ST1 = free recall of a 57-unit story, ST2 = delayed recall of the same story after 48 h; PAL = word paired-associates learning; AVL = auditory verbal learning (Rey, 1964), RM = remote memory. + = unimpaired performance (above the 5% cut-off of a clinical control group without brain damage) − = impaired performance (below the 5% cut-off).

TABLE 3 ASSESSMENT OF VERBAL MEMORY AND LEARNING (GROUP COMPARISONS)

Test		*Group 1*	*Group 2*	*Group 3*	*Controls*
DS	x̄	6.2	6.5	6.4	6.4
	SD	1.2	0.9	1.0	1.1
WL	x̄	5·1	5·6	6·4	6·9
	SD	1·2	0·7	0·9	1·3
ST1	x̄	20.1	28.0	29.3	33.5
	SD	8.5	7.3	10.2	10.7
ST2	x̄	10.0	20.6	23.8	26.1
	SD	8.2	4.2	10.4	11.4
PAL-Trial 1	x̄	0.2	3.5	4.5	4.7
	SD	0.4	2.0	2.3	1.4
PAL-Trial 4	x̄	1.2	6.9	7.6	7.6
	SD	1.1	1.4	0.7	0.8
AVL-Trial 1	x̄	5.2	5.8	6.9	7.0
	SD	1.3	1.3	1.4	1.8
AVL-Trial 5	x̄	8.0	12.8	12.9	13.6
	SD	1.5	1.7	1.5	1.6
AVL-Trial 6*	x̄	3.1	10.8	11.3	11.6
	SD	2.1	2.1	2.7	2.6

For abbreviations, *see* Table 2. Significant group differences ($P < 0.01$) are indicated by braces. * Recall following an interpolated word list.

that all patients in group 1 had a realistic view of their memory deficits. Their complaints especially referred to problems in learning new routes and names or retrieving old ones; patients found it difficult to remember appointments and to store complex verbal information; they felt depressed at their poor performance.

Obvious verbal memory and learning deficits could not be demonstrated either in the 8 remaining patients with left-sided PCI (Cases 13–20; group 2) or in the 10 patients with right-sided PCI (Cases 21–30; group 3). Interestingly, some of the patients in group 2 also complained of an, albeit slight, deterioration in their memory and learning abilities following the infarction.

The diagnosis of memory and learning deficits (*see* Table 2) were based on a rather coarse cut-off criterion of 5%, leading us to search for more subtle differences in test performance. Statistical analysis (two-tailed U tests) comprised data of the 3 PCI groups and of 20 patients without brain damage who were matched with respect to age and educational level (control group). The 0.01 confidence level was adopted for all statistical tests. The results are summarized in Table 3.

In all tests except for the digit span, performance of group 1 was significantly inferior to that of the control group. Results were especially poor on both learning tasks, although there was improvement over trials, as evidenced by a significant increase (Wilcoxon tests) from the first to the last trial. On most tasks performance of group 2 was slightly worse than that of group 3 and the control group. However, only recall of the 10-word list was significantly inferior to controls. Group 3 showed results comparable to controls in all verbal memory and learning tasks.

NEURORADIOLOGICAL EXAMINATION

Scans were performed on a General Electric 9800 CT Scanner. Slices (5 mm) were obtained parallel to the fronto-occipital (FO) line according to Tokunaga *et al.* (1977). This reference line is defined by the frontal and occipital poles of the longer hemisphere, which in most cases is the left and can be determined in a scout view. Within narrow limits the FO line is parallel to the anterior-posterior commissure line (Tokunaga *et al.*, 1977) which is the reference line for the neuroanatomical atlas of Talairach and Szikla (1967) and Nieuwenhuys *et al.* (1980).

Proper localization of the CT lesions was attempted using identifiable landmarks such as the hippocampal and collateral sulci, the transverse fissure, the collateral eminences and various parts of the lateral ventricles. Involvement of the hippocampus (proper) was suggested when the area of infarction extended anteriorly as far as the transverse fissure and laterally to the inferior horn of the lateral ventricle. Infarction areas of all 30 patients were transformed on to 4 horizontal sections from the Talairach and Szikla (1967) atlas. Sections through the FO line, as well as 5 mm and 12 mm below and 8 mm above the FO line, were used.

NEURORADIOLOGICAL RESULTS

CT scans of our patients revealed selective hypodense lesions in the territory of the PCA, defined by sharp outlines and density values as for CSF, indicating a chronic stage of infarction.

With reference to the subtypes of PCI (for explanation, *see* Table 1) there was no essential difference between groups 1 and 3. In both of these groups, subtotal mediobasal infarction as well as infarction in the territory of temporal branches of the PCA occurred. The largest areas of infarction covered almost the entire cortical territory of the PCA; the smallest constituted white matter lesions in the territory of the middle temporal artery. In contradistinction to group 2 (left-sided PCI without memory and learning dysfunction), in group 3 (right-sided PCI without memory and

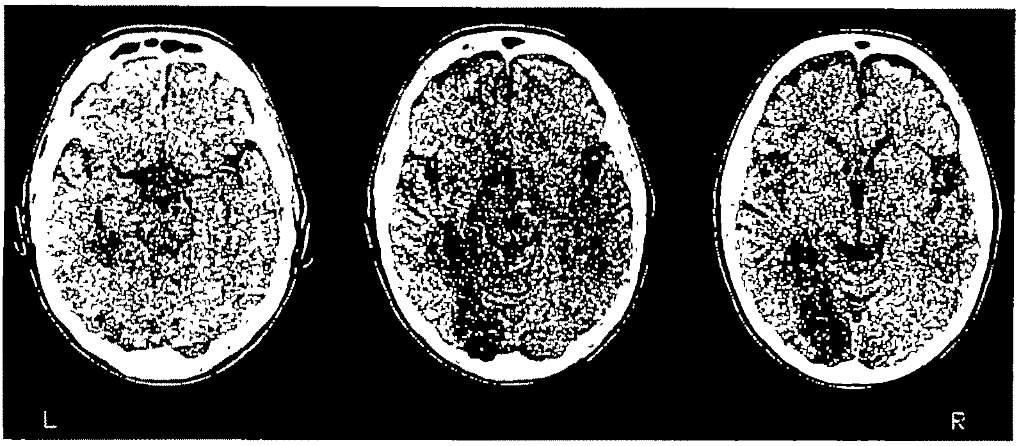


FIG. 2. Three axial CT sections through the region of interest in *Case 7* (female, 58 yrs) with left-sided mediobasal posterior cerebral infarction are shown The infarction area involves the territories of the temporo-occipital artery, the middle temporal artery and the parahippocampal branches. The region around the left hippocampal fissure (hippocampal formation) adjacent to the ambient cistern appears to be undamaged in this case according to CT criteria

learning dysfunctions), there were no parieto-occipital or selective calcarine infarctions. This presumably explains why the common lesioned area in group 3 did not include that of group 2 (*see* figs 3B, C).

In patients with left-sided PCI and severe learning deficits, the *temporomesial* territory of the PCA was involved without exception. The lesioned area common to all 12 patients in group 1 was located around the collateral sulcus in its posterior temporal portion, involving the CI and the PPH in all cases (fig. 3A). The retrosplenial cortex was affected in 7 cases and the hippocampus itself probably in 3 cases (in 4 cases the hippocampus proper may have been partially damaged).

Contrary to this observation, the temporomesial territory of the PCA was mostly spared in all patients in group 2. This also applied to the 3 patients who had a left-sided PCI of the mediobasal type (*see* Table 1). All patients with calcarine or

TABLE 4 'MEMORY RELATED' BRAIN STRUCTURES AFFECTED IN UNILATERAL PCI

Group	*H*	*PPH*	*RSP*	*CI*
1 (n = 12)	3 (4)	12	7	12
2 (n = 8)	0	0 (3)	3	0
3 (n = 10)	6 (1)	8 (1)	7 (1)	9

H = posterior part of the hippocampus proper, PPH = posterior part of the parahippocampal gyrus; RSP = retrosplenial cortex, CI = collateral isthmus Numbers of cases with incomplete lesions are indicated in brackets parieto-occipital infarction belonged exclusively to group 2. The area of overlap was located more posteriorly, involving the region around the calcarine sulcus and the collateral sulcus in its occipital portion (fig. 3B). Even extended infarctions in this group spared the hippocampus and the CI. Lesions could not be detected in CT scans 12 mm below the FO line. The PPH might have been involved in 3 cases, and the retrosplenial cortex was definitely lesioned in 3 others; in 2 patients both brain structures were at least partly damaged.

Table 4 summarizes the data on the 'memory-related' brain structures most likely to have been involved in PCI.

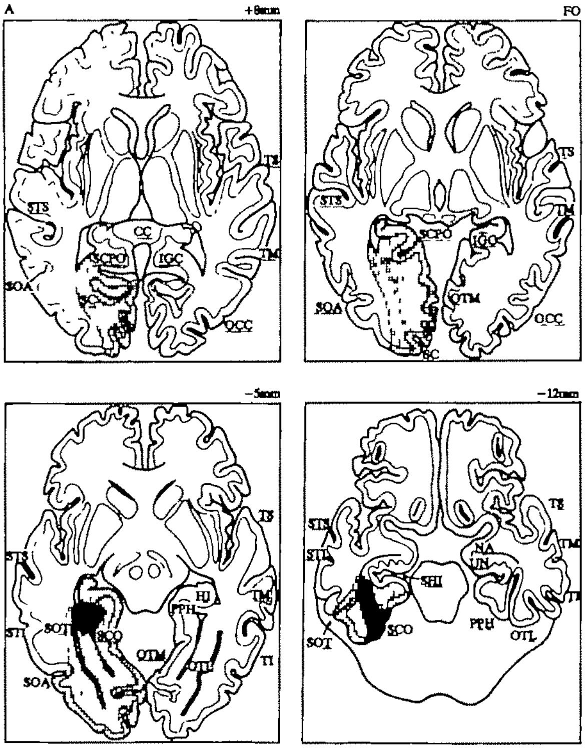
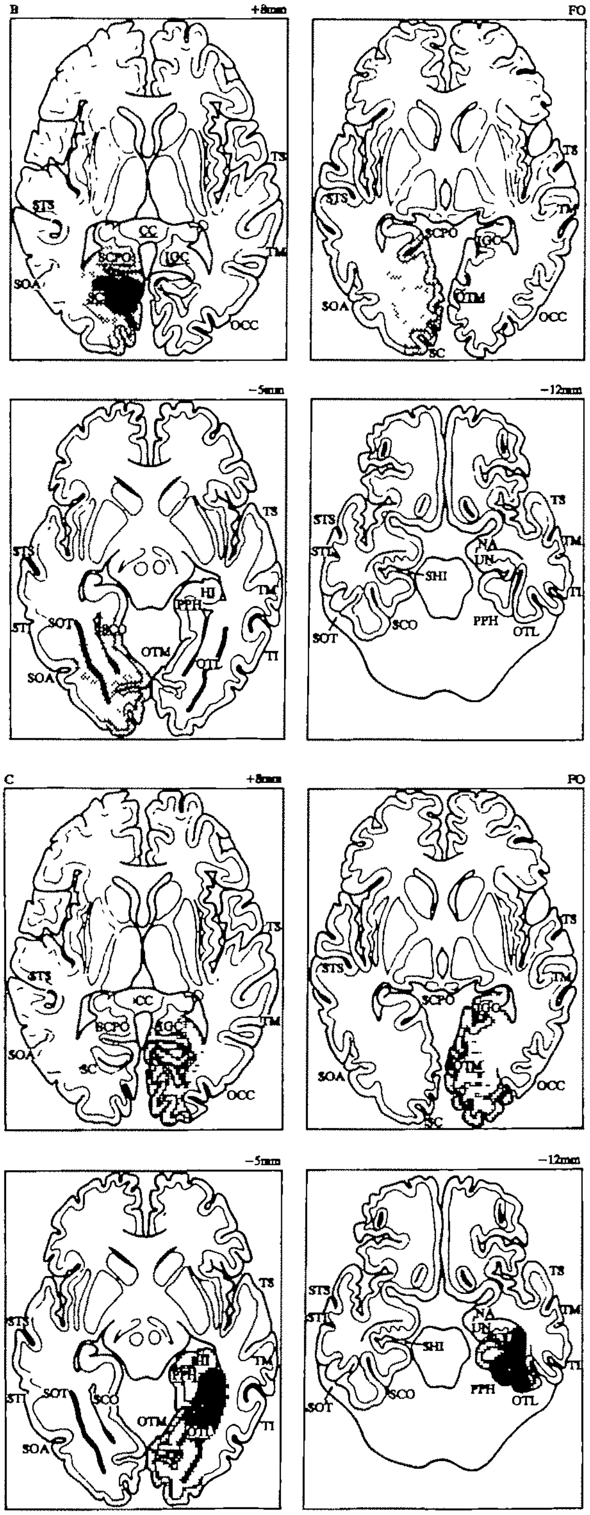


FIG. 3 The lesions of the three groups of patients (*see* below) were transformed separately for each group on to 4 horizontal plates The light grey area covers cerebral structures which are lesioned in at least 1 case of the corresponding group. The lesion area common to all cases of one group is displayed in dark grey The number in the right corner of each plate indicates the level of the plate referred to the FO plane. A, group 1: significant memory and learning deficits in 12 patients (*Cases 1-12* in Table 1) with left-sided posterior cerebral infarction; B, C on facing page. B, group 2: no obvious memory and learning deficits in 8 patients (*Cases 13-20*) with left-sided posterior cerebral infarction, C, group 3, no obvious memory and learning deficits in 10 patients (*Cases 21-30*) with right-sided posterior cerebral infarction.

CC = corpus callosum; IGC = isthmus of the cingulate gyrus; HI = hippocampus; NA = amygdaloid nucleus; OCC = lateral occipital gyri; OTM = medial occipitotemporalis gyrus; OTL = gyrus occipitotemporalis lateralis; PPH = posterior parahippocampal gyrus; TI = inferior temporalis gyrus; TM = temporal medial gyrus; TS = superior temporal gyrus; UN = hippocampal uncus; SC = calcarine sulcus; SCO = collateral sulcus; SCPO = calcarino-parieto-occipital sulcus (common stem); SHI = hippocampal sulcus; SOA = anterior occipital sulcus; SOT = occipitotemporal sulcus; STI = inferior temporal sulcus; STS = superior temporal sulcus.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DISCUSSION

Summarizing the results of memory and learning performance in our 30 patients with unilateral PCI we can conclude that (1) certain patients with left-sided PCI (*see* below) had severe and lasting deficits in verbal memory and learning tasks; (2) another group of left-sided PCI patients displayed only a slight tendency to reduced performance; and (3) patients with right-sided PCI did not differ from controls in any of our verbal memory and learning tasks.

Our observations provide a strong argument against the assumption that severe mnestic dysfunction requires *bilateral* lesions of memory-related brain structures, as claimed by Woods *et al.* (1982).

There seems to be no doubt that verbal memory functions are strictly lateralized. Severe deficits may be expected only in patients whose PCI comprises the temporomedial supply area of the left PCA. The ambidextrous patient (Case 10) also conformed to this rule.

It has been a question of debate as to whether unilateral PCI may yield lasting amnesia. Caplan and Hedley-Whyte (1974) reported on a patient with severe memory disturbance lasting for 6 months related to a left-sided PCI. Their patient had a profound disturbance of 'recent' memory. 'She could recall having seen the examiner before, but never recalled his name or where she had seen him after scores of meetings. At best, she was able to recall one or two facts in a ten-part story after 3 minutes, but seldom recalled any of the story after 10 minutes' (p. 252). Immediate and (as far as tested) long-term retention of isolated and complex verbal material gradually improved within the observation period. The authors noted, however, that the patient never regained memory of the onset of her illness. Learning abilities had not been tested at all. It is therefore clear that this case does not provide satisfactory evidence to support the assumption that memory disturbance is temporary in unilateral left-sided PCI.

Three of our own patients (Cases 3, 6, 9) still showed severe memory deficits approximately 1 year after the brain infarction. It is thus reasonable to assume that *lasting* verbal memory deficits may occur with PCI of the dominant hemisphere. Compared with bilateral PCI, however, these deficits may be less severe. Since the period of observation was less than 1 year for most of our patients, it is possible that in some cases memory functions might improve.

Our group 1 patients were significantly impaired in tasks considered to assess auditory verbal long-term memory. Most prominent was their failure in both learning tasks. Less impairment was seen in story recall, where performance is facilitated by semantic context. Free recall of a word list was significantly reduced in groups 1 and 2 as compared with controls, indicating, in addition, slightly impaired performance for short-term memory. As digit span was normal in all patients, it cannot be concluded that there was a general deficit of auditory short-term memory. This agrees with the findings of Warrington *et al.* (1971), who observed modality-specific impairment of the auditory short-term store related to lesions in the area of the supramarginal and angular gyri of the left hemisphere; these gyri were spared in our cases.

It seems that at least some patients were impaired for the processing of nouns and perhaps other verbal information. A more detailed examination of verbal short-term memory, including the effects of category, frequency and length of words on the recall performance, might reveal that verbal short-term memory is not spared 'a priori' in patients with left-sided PCI.

The slight deterioration of memory and learning abilities of group 2 as compared with group 3 and the control group is not surprising. It can be assumed that these 'minor' verbal memory disturbances are due to small ischaemic lesions of some temporomesial structures (related to verbal memory processing), not visible on CT, which in contrast to right-sided PCI, are extremely likely to occur in all subtypes of *left-sided* PCI. On the other hand, our data indicate that only a substantial infarct (i.e., with damage to these brain structures visible in CT scans, *see* below) causes severe and lasting memory and learning dysfunction.

Patients with *left-sided* vascular lesions in the polar region of the thalamus revealed comparable results in verbal learning tasks as the 12 patients of group 1. Comparing Case 4 of our study on 'thalamic amnesia' (von Cramon *et al.*, 1985), word paired-associates learning as well as auditory verbal learning remained impaired up to 26 months after the brain event. In contrast, disturbed immediate recall of a short word list and a specific retrieval deficit, such as for well-known names, were not observed in the thalamic group.

Compared with bilateral temporomesial or thalamic lesions (von Cramon *et al.*, 1986), patients with unilateral PCI have a thoroughly realistic view of their cognitive problems. The memory disturbance seems to be the main source of their complaints, and for their depressed mood, more than any other neurological or neuropsychological deficit. As mnestic dysfunctions represent a strong 'psychosocial' stressor, this type of depression may be classified as a depressive adjustment disorder (American Psychiatric Association, 1980), which, based on current experience (D. Y. von Cramon, unpublished data), gradually improves with time.

For decades, questions have been raised concerning the role of temporomesial brain structures in chronic amnesia. Repeatedly, the hippocampus has been debated as a critical structure in the medial-temporal-diencephalic memory circuit (*see* Woods *et al.*, 1982). This discussion as to whether hippocampal lesions are sufficient to cause lasting amnesia or not restricts the whole problem to only one aspect. In the clinical literature we know of no case report where *selective* (i.e., exclusive) hippocampal lesion(s) could be held responsible for memory deficits. Also, in the case of Woods *et al.* (1982), there were additional lesions involving the parahippocampal gyrus (PHG) and the CI, at least on the left. Assumed that a left-sided PCI is sufficient to cause lasting (verbal) memory and learning deficits, the authors' line of argument is no longer convincing, as these deficits may be due solely to the left-sided lesions; moreover, the possibility that so-called 'nonverbal' memory tasks were impaired in this case as a consequence of poor verbal strategies for their performance, cannot be excluded.

With our present investigation based on CT evidence we are not able to contribute to the discussion on the role of the hippocampus, because—given the actual resolving power of CT—we cannot guarantee whether this structure was affected or not. What we can affirm with sufficient certainty is the involvement of the PPH and of the CI. As stated above, these structures were both damaged in patients of group 1. The question remains as to what these lesions actually mean to the memory and learning deficits in left-sided PCI.

PPH connections in monkeys and most probably also in man are rather unique. The PPH is a major recipient both of sensory-specific and multimodal association area projections, and a target for intrinsic projections within the limbic lobe. Projections from areas 19 and 20 are visual association projections, thoses from area 22 are auditory association projections, and those from the inferior parietal lobule (area 7) are visuosomatic. Frontal association areas (areas 9 and 46) and the multimodal association area at the banks of the superior temporal sulcus also project to the PPH (Pandya and Kuypers, 1969; Seltzer and Pandya, 1976, 1984; Seltzer and Van Hoesen, 1979). Of equal interest is the fact that there are strong projections back to the association cortices (Van Hoesen, 1982). The projections from the PPH to these cortical areas are extremely widespread. On the other hand, they have to be funnelled through the bottle-neck between the collateral sulcus and the floor of the lateral ventricle on their way to and from the PPH.

A lesion within the CI will thus interrupt bidirectional connections to the PPH with widespread cortical association areas. A combined lesion of the CI and the PPH would be especially devastating; such a lesion deprives the hippocampal formation of its most essential afferent source (from the parahippocampal gyrus via the perforant pathway) and also impairs the neocortical dissemination of hippocampal output. Although routes other than through the parahippocampal gyrus may be taken (via fimbria-fornix and cingulum), there is reason to believe that the projections funnelling through the CI might be of pivotal importance for the interaction between hippocampus/parahippocampal gyrus (as an interconnected unit) and those sensory-specific and multimodal association areas which represent current sensory experiences. If the hippocampus/parahippocampal gyrus no longer has access to the ongoing integrated cortical activity of one hemisphere, it is reasonable to assume that learning (in all modalities?) should be affected. As the left 'verbal' hemisphere is affected, learning by verbal means (strategies) may be primarily impaired.

It is worth noting that in some cases of unilateral PCI, the fimbria-fornix route may also be impassable when damaged by an additional infarction in the territory of the lateral branch of the posterior choroidal artery. Furthermore, damage to the retrosplenial cortex and/or interruption of the cingulate bundle may play a role in mediating memory functions. Information travels via the retrosplenial cortex between anterior (and lateral dorsal) thalamic nucleus and the hippocampus/

parahippocampal gyrus (Rosene and Van Hoesen, 1977), the former being part of the memory-related Papez circuit. It is known that the cingulate bundle is also a constituent of this circuit. Both structures may be selectively damaged in calcarine or parieto-occipital infarction. There were 3 patients in group 2 who actually had sufficiently large lesions of the retrosplenial cortex (and the cingulum?) without obvious memory and learning deficits.

As Mishkin (1982) emphasized, monkeys with bilateral combined amygdalo-hippocampal removals had severe deficits in an object recognition task, whereas animals with either bilateral amygdala or hippocampal excisions revealed merely minor deficits. Concerning the neural mechanisms of memory, the amygdala *and* hippocampus may participate in it equally. In other words, a complete lesion of both structures is the prerequisite for severe and lasting amnesia. Within this concept, lasting verbal memory and learning deficits in unilateral PCI could depend on a lesion interrupting the Papez circuit (by damage to the hippocampus, the fornix, the PPH or the CI) *and* bidirectional projections to and from the amygdala. Bidirectional projections connecting the amygdala with the hippocampus use the PPH via the entorhinal area as a main relay (Van Hoesen, 1982). These connections may be interrupted when the infarcted area, as it did in patients in group 1 (and in group 3), reaches the subcortical white matter of the parahippocampal gyrus.

These considerations should be contrasted with the findings of Gonser *et al.* (1986). They tested memory and learning abilities of epileptic patients who underwent a selective amygdala-hippocampectomy (Yasargil *et al.*, 1985). No obvious memory deficits could be detected after a left-sided excision. Fig. 15 in the paper of Yasargil *et al.* gives a likely explanation for these negative results. It indicates the posterior limits of the approximate incision for hippocampectomy and parahippocampectomy and shows that the posterior parts of the hippocampus/parahippocampal gyrus and the CI are spared with this surgical technique. Thus these findings corroborate the main result of our study, namely that the PPH (and the posterior parts of the hippocampus) with its bidirectional projections funnelling through the CI are *primarily* the critical structures in mediating (verbal) memory and learning functions in monkeys and man. It remains open to be established whether the additional interruption of connections between those structures and the amygdala complex may contribute to the severity and duration of the mnestic disturbance.

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CHOLINERGIC 'BLOCKADE' AS A MODEL FOR CHOLINERGIC DEPLETION

A COMPARISON OF THE MEMORY DEFICITS WITH THOSE OF ALZHEIMER-TYPE DEMENTIA AND THE ALCOHOLIC KORSAKOFF SYNDROME

by M. D. KOPELMAN *and* T. H. CORN[1]

(*From the Institute of Psychiatry, London*)

SUMMARY

The effect of cholinergic 'blockade' on human memory performance as a model for the effect of cholinergic depletion in clinical disorders was investigated. A wide range of memory functions was assessed in 70 subjects, using tests which were identical or closely similar to those which have previously been employed in clinical studies of Alzheimer and Korsakoff patients. In addition, a physiological measure of the degree of central cholinergic blockade was included, as well as measures of subjective arousal and objective attention. It was found that cholinergic blockade had no significant effect on the more passive aspects of primary (or 'working') memory, namely span tests and a measure of verbal short-term forgetting; in this, it contrasts with the marked deficits seen in Alzheimer-type dementia. On the other hand, cholinergic blockade produced impairment at a visuospatial short-term forgetting test, and at a verbal test in which the distractor task was made more difficult. On tests of secondary memory, cholinergic blockade produced a pattern similar to that seen in the anterograde amnesia of Alzheimer and Korsakoff patients, namely a pronounced impairment in learning verbal and visuospatial material, a 'normal' forgetting rate once learning had been accomplished, and relative preservation of the response to priming and of skill learning (procedural memory). Cholinergic blockade, however, did not produce a retrograde amnesia, nor did it affect the recall of temporal context or of long-established semantic knowledge. This pattern of results is compared with that obtained in previous studies of Alzheimer and Korsakoff patients.

INTRODUCTION

Three types of evidence have suggested that cholinergic depletion might play an important role in producing memory impairment. Neuropathological studies have revealed that cholinergic enzymes and synthesis are depleted in Alzheimer and related dementias (e.g., Rossor *et al.*, 1984). Pharmacological studies have demonstrated that cholinergic blockade produces amnesia in animals and man (e.g., Ridley *et al.*, 1984; Kopelman, 1986), thereby confirming the earlier, clinical

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Correspondence to: Dr M. D. Kopelman, Academic Department of Psychiatry, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RP.

[1] Present address: Director of Clinical Neuroscience, Merck Sharp & Dohme Neuroscience Research Centre, Harlow, Essex CM20 2QR.

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observations of anaesthetists (e.g., Hardy and Wakely, 1962). Clinical trials have suggested that administration of cholinergic agonists may occasionally produce a partial alleviation of the memory impairment in Alzheimer-type dementia (e.g., Christie *et al.*, 1981; Little *et al.*, 1985). However, the apparent uniformity of these findings may be deceptive: in general, studies of Alzheimer-type dementia, cholinergic blockade, and clinical trials have tended to use very different types of memory assessment. In order to establish the extent to which cholinergic depletion might account for the memory deficits of Alzheimer-type dementia, it is necessary to compare the effects of cholinergic blockade and dementia on performance at identical or closely similar tests.

An equivalent argument applies, of course, to those other disorders in which it has also been postulated that memory impairment might be attributable to cholinergic depletion, for example, Parkinson's disease, Down's syndrome and the alcoholic Korsakoff syndrome. In the last disorder, there is some evidence that thiamine depletion may inhibit acetylcholine synthesis (Witt, 1985) and that cholinergic neurons in the basal forebrain may be reduced in number (Arendt *et al.*, 1983), although it remains to be confirmed whether the latter abnormality is indeed characteristic of the specific amnesic (Korsakoff) syndrome, a more global alcoholic dementia (Lishman, 1986), or only a subgroup of these patients.

Recent research has postulated a number of subcomponents in memory processes, and these can be differentially impaired in dementing and amnesic disorders (*see e.g.*, Baddeley, 1984; Weiskrantz, 1985; Warrington, 1986; Kopelman, 1987*a*). These various memory subcomponents have seldom been examined in detail in cholinergic blockade studies (Kopelman, 1986), and the present investigation sought to examine these processes, using tests derived from neuropsychological studies. The principal concepts to be considered are as follows.

1. *Primary or working memory.* This refers to tasks or processes in which small quantities of information are recalled over short retention periods, not longer than approximately 30 s. It is severely impaired in Alzheimer-type dementia (Miller, 1973; Corkin, 1982; Kopelman, 1985; Morris, 1986), but is often unaffected in the Korsakoff syndrome, although there is some disagreement about this (*see* Baddeley and Warrington, 1970; Butters and Cermak, 1980; Kopelman, 1985).
2. *Secondary memory—anterograde ('explicit') component.* This refers to the learning, retention and retrieval of information held in memory for periods longer than approximately 30 s. Both amnesia and dementia are, of course, characterized by a severe impairment in learning verbal and visuospatial material, whether tested by recall or recognition (e.g., Miller, 1973; Corkin, 1982; Warrington, 1986). After learning has been accomplished its 'forgetting rate' can be measured. If special measures are taken to overcome the severe learning deficit in amnesic and dementing disorders, there is evidence that the forgetting rate of new information is normal (Huppert and Piercy, 1978*a*; Kopelman, 1985; Martone *et al.*, 1986; Becker *et al.*, 1987; Freed *et al.*, 1987).

3. *Secondary memory—'implicit' component*. This refers to learning of which the subject may not have 'conscious' awareness. Examples of this are (1) *'priming'*, that is, the facilitation of a particular response following exposure to a specific stimulus, even though the original stimulus may itself be neither recalled nor recognized; the response to priming is often spared in the amnesic syndrome (Warrington and Weiskrantz, 1970; Graf *et al.*, 1984; Shimamura, 1986), although not necessarily in dementing disorders (Kopelman, 1987*b*); (2) *skill learning* (*'procedural memory'*), which is also spared in the amnesic syndrome and in some Alzheimer patients (Cohen and Squire, 1980; Martone *et al.*, 1984; Knopman and Nissen, 1987).

4. *Secondary memory—'semantic'*. This refers to memories for facts, concepts and language, as opposed to memory for personal events or experiences. Some of this knowledge will have been established long before the onset of amnesia or dementia. There is evidence that certain aspects may be spared in the amnesic syndrome although impaired in dementia (Baddeley, 1984; Warrington, 1986).

5. *Secondary memory—retrograde component*. There is an extensive retrograde loss in both Alzheimer-type dementia and the Korsakoff syndrome, as assessed by 'remote memory' tests, although there is disagreement about the degree of 'sparing' of early memories in these disorders (Sanders and Warrington, 1971; Albert *et al.*, 1979; Weiskrantz, 1985).

The present paper reports findings in a study of the effect of cholinergic blockade on these various aspects of memory, using cholinergic blockade as a model for cholinergic depletion. The results will be compared with those obtained in previous studies of Alzheimer-type dementia and the Korsakoff syndrome. On the basis of previous studies reviewed elsewhere (Kopelman, 1986), it was hypothesised that, while the pattern of memory impairment seen in the anterograde ('explicit' and 'implicit') components of secondary memory might indeed be consistent with that seen in the Alzheimer and Korsakoff syndromes, cholinergic depletion cannot account for deficits of primary/'working' memory, long-established aspects of semantic memory, or remote memory.

OVERALL STRATEGY

The study was conducted in two phases (Table 1). In the first experiment, the effect of hyoscine (scopolamine) on various measures of primary or working memory was examined, as well as its effect on various tests of 'short-term' attention. The order of test administration was varied, and a dose-response relationship was sought. In the second experiment, the effect of hyoscine was investigated with respect to tests of verbal and visuospatial learning, measures of the forgetting rate and tests of remote, implicit and semantic memory. The effect of manipulating the difficulty of a 'distractor task' between stimulus presentation and recall was also studied. On the basis of the results in the first experiment, only the higher dose of hyoscine was administered in the second, and the most sensitive measure of short-term attention was readministered. In both phases of the study, measures of subjective arousal and the physiological degree of cholinergic blockade were included. A covariance analysis was conducted to try to determine the relative contribution of physiological

TABLE 1 PROCEDURE FOR EXPERIMENTS 1 AND 2, DAYS 1 AND 2

Time	*Experiment 1*	*Experiment 2, day 1*	*Experiment 2, day 2 (48 h delay)*
−5 min	Arrival. Clinical room (NART, near-point, arousal)	Arrival: Clinical room (NART, near-point, arousal)	Arrival: Clinical room (NART, near-point, arousal)
0 min	Drug/placebo ↓ (near-point, arousal)	Drug/placebo ↓ (near-point, arousal)	Drug/placebo ↓ (near-point, arousal)
30 min	Experimental lab.: Tests of 'attention' and 'primary memory' (Order of tests systematically varied): Simple RT Digit span Verbal short-term forgetting	Experimental lab.: H-P slide exposure (= 3 min at 0.5 s/slide = 5 min at 1.5 s/slide) ↓ PAL test ± simple/choice RT (10 min) ↓	Experimental lab.: Recognition test 3: (10–12 min) followed by H-P recall test: 'today' and 'previous' + delayed recall tests: Logical memory Cued list recall PAL Skill learning and word recall
43 min	Block span Visuospatial short-term forgetting Stroop PASAT Simple/choice RT	Recognition test 1: (10–12 min approx. followed by simple/choice RT in condition 4) ↓	+ Verbal fluency (F,A,S) Distractor variation test Remote memory test
60 min	↓	Clinical room: (Placebo in PP, DD groups Drug infusion in PDP group, followed at 30 min by near-point, arousal) ↓	↓
95–100 min (approx.)	Clinical room: Near-point, arousal. End.	↓	Clinical room: near-point, arousal. End.
105 min		Experimental lab.: 'Learning tests': Logical memory, word list recall, object learning, skill learning ↓	
150 min		Clinical room ↓	
195 min		Experimental lab.: Recognition test 2 (10–12 min) ↓	
205 min (approx.)		Clinical room: near-point, arousal. End.	

H-P = Huppert-Piercy.

blockade, subjective arousal and an objective measure of 'short-term' attention to the principal findings on memory tests.

Hyoscine was administered by intravenous infusion in order to ensure a rapid and adequate uptake into the central nervous system (CNS). After some consideration, it was decided to employ a saline placebo. Of the peripheral cholinergic antagonists, methoscopolamine has not been employed in earlier studies using intravenous administration because of its potential side effects, and there is some evidence that glycopyrrolate may be partially absorbed into the CNS (Proakis and Harris, 1978), the cognitive effects of which have largely been unexplored An important aspect of the study was to determine which attentional and memory processes remained relatively unaffected by cholinergic blockade; the use of an inert placebo minimizes the risk that any negative findings will be spurious. Moreover, the possible determinants of positive findings could be scrutinized by the use of a correlational and covariance analysis. A parallel group design was employed in order to make the study and the statistical analysis as closely similar as possible to investigations in patients and controls. We were primarily interested in making inferences from the drug studies to patient studies, rather than in eliciting subtle pharmacological effects of hyoscine. Furthermore, this strategy avoided the need to give repeated injections and tests to the same subjects.

The physiological index of central cholinergic blockade employed in this study was the change in visual near-point which followed drug administration. Systemic hyoscine produces pronounced ocular effects whereas atropine or the quarternary ammonium compounds do not (Weiner, 1980) indicating that this property is closely correlated with the 'central' actions of the drug. Measurements were taken preinfusion, immediately pretesting and at the completion of testing. The difference between the first reading and the mean of the latter 2 readings was used as an index of the degree of cholinergic blockade obtained during psychological testing. Subjective arousal was rated by the subjects on a visual analogue scale. These ratings were made at the same intervals as visual near-point was determined, and a difference score obtained in the same way.

Statistical analysis was by ANOVA or t tests (as appropriate) for independent groups, except when a comparison was made of the same subjects' performance on a verbal and a nonverbal test where a repeated measures design was used. The Kruskal-Wallis nonparametric ANOVA was used to examine error patterns on one of the tests. Product moment correlations were employed to examine the degree of association between performance on the different tests.

Subjects were healthy volunteers recruited from the staff (unskilled, secretarial and professional) of the Wellcome Research Laboratories. Before inclusion in the study, they were interviewed and a physical examination and an ECG were performed. Their general practitioners were notified of intended participation. Exclusion criteria were (1) any complaint of memory impairment, or a history of severe or recent head injury or heavy alcohol intake; (2) a history of cardiovascular or respiratory pathology; (3) glaucoma or prostatic symptoms. Informed consent was obtained and the study was approved by the ethical committees of the Institute of Psychiatry and the Wellcome Research Laboratories.

EXPERIMENT 1

In this experiment, the effect of cholinergic blockade on tests of 'short-term' attention and primary or working memory was investigated.

By 'short-term attention', we refer to tasks performed over a period of approximately 1 to 5 min, so that they are comparable with most of the learning tests described below, rather than tests of 'vigilance' conducted over more extended periods. A difficulty in selecting such tests of 'short-term attention' is the low intercorrelation between different tasks, reflecting the varying processes involved (Broadbent, 1981). In the present study, 'single channel' tasks were employed (involving a single source of stimulus input) rather than 'dual channel' tasks (e.g., dichotic listening) to make the tests comparable with the memory tests, which also entailed a single channel of stimulus input. The tests were ones which have been widely used in previous studies of ageing, brain pathology and psychopharmacology, namely the Stroop test, PASAT (Paced Auditory Serial Addition Task) and simple and choice reaction time (RT) (e.g., Gonwall and Wrightson, 1974; Thompson *et al.*, 1981; Nuotto, 1983).

The tests of primary/'working' memory were measures of verbal (digit) and visuospatial (block) span, whose impairment has traditionally been interpreted as reflecting diminished primary memory capacity (e.g., Miller, 1973; Butters and Cermak, 1980), as well as tests of verbal and visuospatial, 'short-term' forgetting. Visuospatial span and short-term forgetting have not previously been reported in studies of cholinergic blockade; and a previous study of verbal short-term forgetting (Caine *et al.*, 1981) used a small sample and a somewhat different procedure from that employed in Alzheimer studies.

METHODS

Thirty subjects were recruited from the Wellcome Research Laboratories, as described above. They were allotted to 3 parallel groups in a semistratified fashion to match as closely as possible for age, sex and educational level (Table 2). Subject recruitment and allocation, as well as drug administration and physiological monitoring during the experiment, were carried out by one author (T.H.C.). Test administration was conducted in a separate room by the other author (M.D.K.) who, like the subjects, was blind to the treatment condition.

The experimental procedure is illustrated in the left-hand column of Table 1. The subjects were given an i.v. infusion over 10 min of either 0.4 mg hyoscine, 0.2 mg hyoscine or normal saline and, 30 min after the start of the infusion, were transferred from the clinical room into an experimental

TABLE 2 SUMMARY DESCRIPTION OF SUBJECT GROUPS

Experiment 1 *Group*	*n*	*Mean age (±SD)*	*Sex ratio* *M F*	*NART IQ*[1] *Mean (±SD)*	*Mean near-point change score*[2]
1. Placebo	10	35.7 (±9.7)	4 6	114.4 (±5.7)	0.12 cm
2. Hyoscine 0.2 mg i.v.	10	38.5 (±9.4)	4 6	119.2 (±3.8)	2.21 cm
3. Hyoscine 0.4 mg i.v.	10	40.4 (±11.9)	4 6	115.6 (±7.0)	9.93 cm

Experiment 2 *Group*[3]	*n*	*Mean age (±SD)*	*Sex ratio* *M F*	*NART IQ*[1] *Mean (±SD)*	*Mean near-point change*[2] *Day 1*	 *Day 2*
1 PP	10	28.8 (±7.5)	4 6	114.6 (±9.1)	0.10 cm	0.39 cm
2 DD (blind)	10	28.3 (±8.7)	4 6	112.3 (±8.6)	7.89 cm	9.74 cm
3 PDP	10	30.1 (±9.2)	3 7	109.5 (±6.4)	5.15 cm[4]	0.03 cm
4 DD (nonblind)	10	27.9 (±6.5)	4 6	111.5 (±5.6)	3.35 cm	4.31 cm

[1] NART = New Adult Reading Test (Nelson and O'Connell, 1978) [2] Near-point change score $= \left(\frac{NP2+NP3}{2}\right) - NP1$, where NP1 = reading preinfusion, NP2 = reading immediately pretesting and NP3 = reading at end of testing [3] PP = placebo administered on both days of experiment. DD = drug administered on both days of experiment, i.e., hyoscine 0.4 mg i.v. PDP = placebo followed by drug on day 1, placebo on day 2 [4] Reading for change in visual near-point following hyoscine administration

room in which testing commenced. The first 15 subjects received the tests in one order, and the remaining 15 in the reverse order. Testing was completed 95–100 min after the start of the infusion.

Tests employed

1. *National Adult Reading Test* (Nelson and O'Connell, 1978). This requires subjects to read and pronounce a list of 50 irregularly spelled words from which a quick estimate of IQ can be determined in healthy subjects.

2. *Visual near-point.* Subjects held the end of a tape measure to the tip of their nose. A sample of prose in Times Roman print attached to the body of the measure was moved progressively inward and the subject stated the point at which it became out of focus.

3. *Subjective arousal.* Subjects indicated their current level of subjective arousal along four 10 cm visual analogue lines, which were labelled respectively 'alert'/'drowsy', 'clear-headed'/'muzzy', 'mentally slow'/'quick witted', 'dreamy'/'attentive' (*see* Bond and Lader, 1974). These ratings proved to be highly correlated, and the mean reading was taken to reflect the subject's current level of 'subjective arousal'.

4. *Simple and Choice Reaction Time* (*RT*). The subject faced a semicircle of 8 red lights displayed against a black surface at an angle of 30°, immediately beneath each of which was a green button at a radius of 10 cm from a central button (*see* Miller, 1970). When a light came on the subject was required to put it out by pressing the button underneath as rapidly as possible, returning his/her forefinger to the central button between trials. There were 4 experimental conditions, in which the stimulus was either a single light (simple RT) or one of 2-, 4- or 8-choices (choice RT). There were 5 'practice' and 16 'test' trials in each condition, and reaction time was automatically measured. The simple RT condition was performed twice, once at the beginning and again at the end of the experiment, to see if there were any (drug-related) changes with time.

5. *Stroop Test.* Two colour-naming tasks were used. Each was presented on a 37.5 cm × 37.5 cm white card, consisting of 100 items in 10 rows and 10 columns. In the control condition, each item consisted of a series of 5 Xs (XXXXX) written (0.5 cm high) in a particular colour (red, yellow, green, brown or blue). Each item (colour) appeared twice in each row, never twice in succession (or vertically above the same colour), but otherwise their distribution was random. In the 'conflict' condition, the items were colour names (i.e., the colours used above), which were printed in a *different* colour from that the name described. Subjects were required to state (row by row) the colours in which the Xs or words were printed as rapidly as possible, and their speed and error rate in reading each card was noted.

6 *PASAT* (Gronwall and Wrightson, 1974). Subjects listened to a tape-recorded series of 61 single digits, presented in a random order at a rate of a digit every 2.4, 2.0, 1.6 and 1.2 s in successive trials. The subject was required to add each digit to the one immediately before (the second to the first, the third to the second, etc), giving the answer aloud. The number of correct responses out of 60 was determined for each condition.

7. *Digit and Block Span.* Digit span forward was determined in accordance with the procedure employed in the Wechsler Memory Scale (Wechsler, 1945). Block span was determined using the Corsi blocks, an arrangement of 9 black wooden blocks in an irregular pattern on a black baseboard (Smirni *et al.*, 1983). The experimenter tapped a series of (3 or more) blocks at a rate of 1/s; the subject was asked to tap the same blocks in the same sequence immediately afterwards. 'Block span' was the longest sequence perfectly recalled. There were 2 trials at each sequence length. Sequences of a given length were matched for 'difficulty' by tapping series identified as such by Smirni *et al.* (1983).

8. *Short-term forgetting.* Verbal short-term forgetting was assessed by administering the Brown-Peterson test, using a procedure identical to that employed by Kopelman (1985). Each subject viewed a Telex Caramate back-projector screen. Following a 'Ready' slide, the subject saw a slide showing 3 three-letter words of high (A or AA) frequency (Thorndike and Lorge, 1944), printed white against a black background. This slide was exposed for 2 s and the subject was requested to

read the words aloud. The next slide indicated either a question mark ('?'), the cue for recall ('no delay' condition) or the number '100', the cue to perform a distractor task, which was counting backwards from 100 by threes as rapidly as possible. In the latter condition, the '?' appeared at varying delay intervals (2, 5, 10 or 20 s), at which the subject attempted to recall the words. There were 5 'practice' trials, and 5 'test' trials in the 'no delay' condition and at each of the delay intervals (i.e., 25 'test' trials). These were administered in blocks of 5 trials with the sequence of delay intervals randomized within each block. Presentation time of the slides was fully automated, as was presentation of the cue for recall at 0 and 2 s, but the 5, 10 and 20 s delays were timed manually.

9. *Nonverbal short-term forgetting*. This was tested using a method derived from Sullivan *et al.* (1986). The experimenter touched 3 (randomly selected) Corsi blocks, and the subject was requested to touch the same blocks in the same order and then to replace his/her hands on the table about 30 cm apart. Either the subject was requested immediately to 'recall', that is, to retouch the blocks, the 'no delay' condition, or he/she performed the distractor task of Sullivan *et al.* for 2 to 20 s during which the blocks were covered. The distractor task consisted of the experimenter touching one of the subject's fingers, which the latter then used to touch the same finger on the opposite hand, replacing the hand for the experimenter to touch another finger (on either hand), all the time urging the subject to perform the task as rapidly as possible. As in the verbal test, there were 5 'practice' trials and 5 'test' trials at each of 5 delay intervals (0, 2, 5, 10, 20 s), administered in blocks of 5 trials with the sequence of delay intervals randomized within each block. Both the accuracy and the sequence of the subject's recall responses were noted

RESULTS

Table 2 shows that, although the groups did not differ significantly in terms of either age ($F(2,27) = 0.52$) or IQ as measured by NART ($F = 1.94$), they showed a substantial difference in terms of mean visual near-point change ($F = 32.48$, $P < 0.001$). There was also a very marked difference in this measure between the high and low-dose hyoscine groups ($t = 5.00$, $P < 0.001$, 2t), and between the low-dose and the placebo groups ($t = 4.33$, $P < 0.001$, 2t).

Table 3 shows the results on the measure of 'subjective arousal' and two of the three tests of 'short-term' attention. The mean change in 'subjective arousal', as defined above, showed a shift in the direction of diminished arousal during the

TABLE 3 AROUSAL AND ATTENTION TESTS

	Placebo	*Hyoscine 0.2 mg i.v.*	*Hyoscine 0.4 mg i.v.*
1. Subjective arousal			
Mean change score (cm)	1.05 (±1.13)	2.45 (±1.05)	3.51 (±0.99)
2. Stroop			
Speed (conflict-control) (s)	53.1 (±11.45)	51.95 (±16.0)	57.8 (±14.95)
Errors (conflict-control)	0.7 (±1.25)	1.9 (±2.23)	3.6 (±4.1)
3. PASAT			
Total correct score at			
2.4 s/digit	44.7 (±10.2)	43.1 (±11.1)	39.5 (±18.4)
2.0 s/digit	38.2 (±10.5)	36.5 (±8.5)	33.0 (±11.7)
1.6 s/digit	30.2 (±9.1)	31.4 (±7.1)	25.1 (±9.7)
1.2 s/digit	24.9 (±8.6)	25.7 (±6.7)	21.5 (±7.75)

experiment in all 3 groups, but the difference between the groups was highly significant (F(2,27) = 13.55, $P < 0.001$). The higher dose group manifested a greater effect than the lower dose group (t = 2.32, $P < 0.05$, 2t) which, in turn, showed a more pronounced change than the placebo group (t = 2.86, $P < 0.05$, 2t).

By contrast, Table 3 also shows that there was minimal difference between the groups on two of the three 'objective' tests of short-term attention. On the Stroop test, there was no significant difference between the groups in total response time in either the control (F = 1.57) or conflict condition (F = 0.83) or in terms of the (conflict-control) difference (F = 0.23). There was a trend for the drug groups to give more errors in the conflict condition, but this failed to reach significance either in terms of total errors (H = 2.29) or the (conflict-control) difference (H = 1.51). Similarly, Table 3 shows a trend for the higher dose hyoscine group to give fewer correct responses than the other 2 groups on the PASAT at each presentation rate, but the difference between the groups was not statistically significant in terms of either a main (F(2,27) = 0.77) or (group × presentation rate) interaction effect (F(6,81) = 0.35).

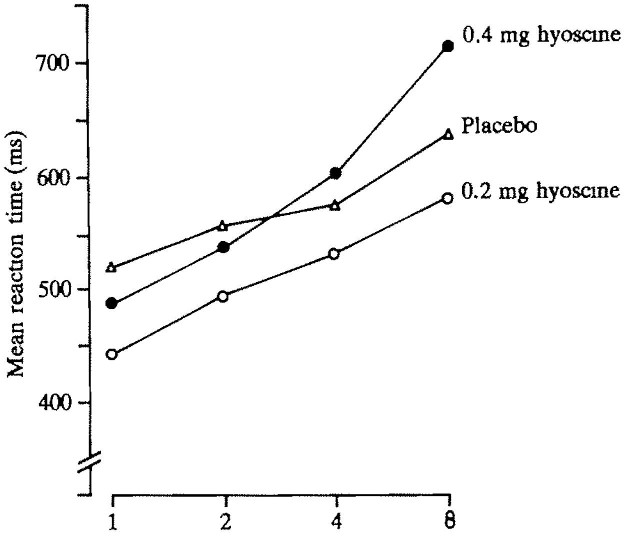

FIG. 1 Simple and Choice Reaction time.

By contrast, fig. 1 shows the pattern of results for the simple and choice reaction time task. In this test, there was a significant main effect of groups (F(2,27) = 4.63, $P < 0.05$) and a significant (group × number of choices) interaction (F(6,81) = 4.64, $P < 0.01$). Inspection of the curves indicates that the low-dose hyoscine group responded *faster* than the placebo group, whatever the number of choices (main effect of groups F(1,18) = 6.75, $P < 0.05$), but that there was no (group × choices) interaction (F = 0.95). On the other hand, comparison of the higher dose and the placebo group indicates that, although there was no overall difference in their level of performance (main effect F = 0.19), there was a very pronounced (group × number of choices) interaction (F(1,54) = 6.68, $P < 0.05$). In short, it appears that, at the higher dose of hyoscine, performance was impaired as the task complexity (number of choices) was increased.

TABLE 4 SPAN TESTS (mean score ±SD)

	Placebo	*Hyoscine 0.2 mg*	*Hyoscine 0.4 mg*	*Significance*
Digit	7.7 (±1.25)	7.2 (±0.9)	7.2 (±0.6)	n.s.
Block	5.3 (±0.95)	5.5 (±0.7)	5.5 (±0.5)	n.s.

Table 4 shows the results on the span tests. There was a slight trend for the 2 drug groups to perform worse at digit span, but this difference was not statistically significant (F(2,27) = 0.61) and it was not evident in the block span result.

Fig. 2 plots the results of the verbal short-term forgetting (Brown-Peterson) test. It shows the mean number of words correctly recalled at each time interval by the 3 groups. It can be seen that there was a trend for the 2 drug groups to recall fewer words at the longer retention intervals (5 s and beyond). However, neither the main effect of drug group (F(2,27) = 1.23) nor the (group × delay) interaction (F(8,108) = 0.99) was statistically significant.

The visuospatial short-term forgetting test could be scored in terms of either correct blocks in any order (analogous to the verbal test) or correct blocks with

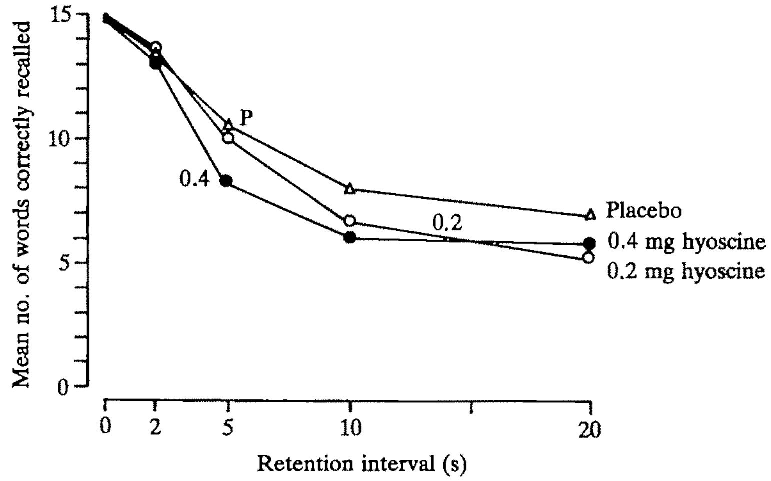

FIG. 2. Verbal short-term forgetting test (Brown-Peterson Test). Distraction task = counting backwards from 100 by threes P = placebo, 0.2

bonus points for correct sequence, or correct blocks in the correct sequence only. In comparing the pattern of performance between the 3 groups, these 3 scoring methods produced closely similar results; but the third method (the hardest criterion) produced a level of performance in the placebo group which was closely similar to that obtained in the verbal test (i.e., similar task 'difficulty') and is illustrated in fig. 3. On this test, there was a significant main effect of groups (F(2,27) = 5.68, $P < 0.01$). This was entirely accounted for by the difference between the high-dose and placebo groups (F(1,18) = 8.91, $P < 0.01$), the performance of the low-dose and placebo groups being closely similar (F = 0.03). It can be seen that the divergence between the performance of the high-dose and placebo groups occurred entirely in the first 2 s; thereafter, their curves remained in parallel and, in fact, the overall (group x delay) interaction effect failed to reach statistical significance (F(4,72) = 2.22). Within each drug group, comparisons can be made of performance at the visuospatial test, scored in this way, relative to the verbal (Brown-Peterson) test. Whereas there was no significant main effect of verbal versus visuospatial material in the placebo group (F(1,9) = 1.53) or low-dose group (F < 0.01), there was a very significant difference in the high-dose group (F(1,9) = 29.09, $P < 0.001$) as well as a significant (delay x test material) interaction effect (F(4,36) = 4.57, $P < 0.01$). These findings confirm that the high-dose group performed worse at the visuospatial test.

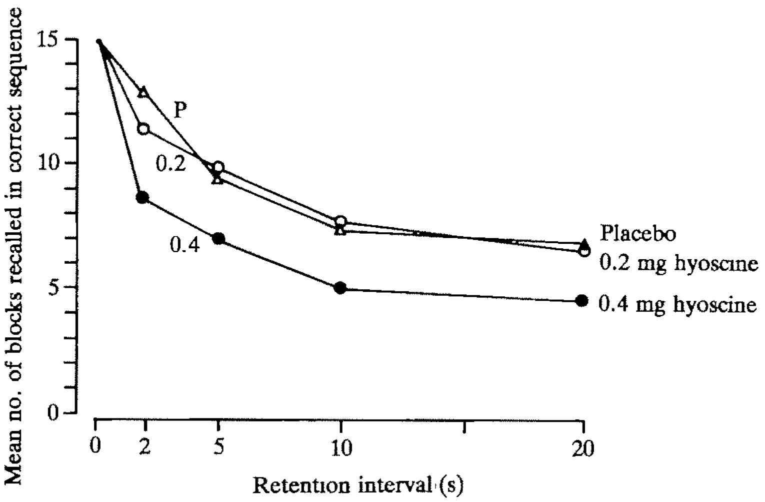

FIG 3. Visuospatial short-term forgetting test (block retention test) Distraction = finger-tapping task (*see* text). P = placebo.

Table 5 provides a correlation matrix between the independent and dependent variables in this experiment. 'RT difference' refers to the mean difference between simple RT and 8-choice RT, and 'span : total' to the sum of digit and block span.

TABLE 5 EXPERIMENT 1 TEST INTERCORRELATIONS (n = 30)

	Age	*IQ*	*Near-point*	*Arousal*	*RT difference*	*Stroop difference*	*PASAT*	*Span total*	*Verbal short-term forgetting*	*Nonverbal short-term forgetting*
Age	1.00									
IQ	−0.03	1.00								
Near-point change score	0.40*	−0.16	1.00							
Arousal change score	0.03	−0.02	0.64***	1.00						
RT difference score	0.13	0.03	0.58***	0.63***	1.00					
Stroop difference score	0.51**	−0.16	0.15	−0.05	0.00	1.00				
PASAT total score	−0.54**	0.29	−0.49**	−0.34	−0.28	−0.05	1.00			
Span (verbal + nonverbal) total score	−0.15	0.06	−0.14	−0.02	−0.05	−0.32	0.05	1.00		
Verbal short-term forgetting (total score)	−0.62***	0.37*	−0.55**	−0.24	−0.24	−0.25	0.60***	0.27	1.00	
Nonverbal short-term forgetting (total score)	−0.77***	0.17	−0.62***	−0.22	−0.36	−0.44*	−0.48**	0.35	0.62***	1.00

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In view of the number of correlations in this matrix, significance values at the 0.05 level need to be interpreted with caution

The significant associations between age, IQ and short-term forgetting confirm the findings of an earlier study of healthy subjects (Kopelman, 1985). The change in visual near-point (the index of cholinergic blockade) correlated significantly with PASAT and verbal short-term forgetting score as well as with 'subjective arousal' change, RT difference score, and visuospatial short-term forgetting. The various 'attention' tests correlated together poorly, and only 'RT difference' was significantly asociated with 'subjective arousal'. The PASAT contains a memory component and correlated positively with both forgetting tasks. Digit and block span performance correlated at r = 0.40 ($P < 0.05$), and total verbal and visuospatial short-term forgetting scores correlated at r = 0.62 ($P < 0.001$).

There was no significant difference between simple reaction time tested at the beginning and the end of the experimental session (paired t = 1.04), and no significant or systematic effects were found with respect to the order of test administration.

DISCUSSION

In the present study, the objective tests of 'short-term' attention correlated poorly together (*see* Broadbent, 1981), and only 'RT difference' showed a significant impairment following administration of hyoscine (at the higher dose). This measure also correlated significantly with the change in 'subjective arousal', and the latter scale appeared to be particularly sensitive to cholinergic blockade. Previous studies have demonstrated an effect of cholinergic blockade on subjective rating scales (e.g., Nuotto, 1983; Parrott, 1986) and tests of vigilance over extended periods (Wesnes and Warburton, 1984). Results in studies of 'objective' attention have been somewhat conflicting, some studies failing to find any effect at doses at which memory has been impaired (Crow, 1979; Caine *et al.*, 1981). Consistent with the present finding, Nuotto (1983) and Parrott (1986) have previously reported increased choice RT following hyoscine administration, and Baddeley *et al.* (1986) have reported increased reaction times to tones in dementing patients.

Consistent with previous studies, cholinergic blockade did not affect digit span (e.g., Drachman and Leavitt, 1974), nor did it produce any impairment of 'block' span. As has been discussed elsewhere (Kopelman, 1986), this absence of effect contrasts with the reduction in span usually seen in Alzheimer-type dementia, the degree of the impairment being proportional to the clinical severity of the dementia (Corkin, 1982). Similarly, cholinergic blockade produced only minimal impairment (not statistically significant) on the test of verbal short-term forgetting, comparable to a finding previously obtained in Korsakoff patients, but in marked contrast with the severe impairment of Alzheimer patients at an identical form of this test (Kopelman, 1985). Performance at the visuospatial 'short-term' forgetting test correlated significantly with scores on the verbal version but, in this case, the difference between the groups was statistically significant. Moreover, the pattern and degree of the impairment (in the 0.4 mg hyoscine group) was similar to that reported by Sullivan *et al.* (1986) in a study of Alzheimer patients. Consistent with the present result, Sullivan *et al.* found that the performance of their healthy controls and Alzheimer patients diverged most markedly during the first 3 s delay. They commented that the 'short-term' retention of nonverbal material may be particularly vulnerable in Alzheimer patients.

EXPERIMENT 2

This experiment investigated the effect of cholinergic blockade upon various aspects of learning and forgetting. Memory for both verbal and visuospatial material was assessed, and the tests were selected for being those employed in previous studies of Alzheimer and Korsakoff patients. A test of remote memory was included to assess any retrograde memory impairment, as well as a test of well-rehearsed semantic memory. In addition, the effect of cueing and aspects of 'implicit' memory ('priming', skill learning) were investigated. Despite great interest in their functioning in amnesic and dementing patients, these latter components of memory have very seldom been examined in studies of cholinergic blockade, an exception being a recent study by Nissen *et al.* (1987) who reported preserved learning on a serial reaction time task. In order to facilitate comparison with the results of Experiment 1, measures of visual near-point, subjective 'arousal' and 'RT difference' were again included.

METHODS

Forty volunteers were recruited from the Wellcome Research Laboratories, some of whom had taken part in the earlier experiment almost a year before but most of whom were new. Subject recruitment and allocation were again conducted by T.H.C., and testing by M.D.K., and the subjects were allocated in a semistratified fashion to 4 parallel groups, matched as closely as possible for age, sex and educational level. Because of chance recruitment factors, the mean ages of the present groups were rather younger than in the first experiment, but Table 2 shows that the 4 groups were closely matched for age (F(3,36) = 0.14).

The present experiment was conducted in 2 sessions, 48 h apart. The overall design of the experiment is illustrated in Table 1. In the first group (PP), subjects received a 10 min i.v. infusion of saline placebo on arrival at the laboratory on both days, and in the second (DD) they received an infusion of 0.4 mg hyoscine over 10 min on both days (*see* Tables 1, 2). In both these groups, the subjects also received an injection of placebo through a butterfly cannula at the 60 min interval on day 1. In condition 3 (PDP), the subjects received a 10 min infusion of placebo on arrival at the laboratory on both days, but this group received a 10 min infusion of 0.4 mg hyoscine at the 60 min interval on day 1. In each of these conditions, M.D.K. and the subject were blind to the drug treatment. However, condition 4 (DD nonblind) was identical to condition 2, except that T.H.C. informed M.D.K. (but not the subject) on day 1 that this subject was in 'condition 4', and the experimental procedure for two of the tests was modified accordingly (*see* below). Because of the absence of order effects in the previous experiment, and the complexity of the design, tests were administered in the same order for all subjects (with one small variation, mentioned below). The design was such that 2 subjects could be tested alternately in a given session on day 1.

Tests employed

1. *NART, visual near-point, subjective arousal, simple and 8-choice RT.* These tests were administered as above. Simple and 8-choice RT ('RT difference') were determined between slide exposure and the first recognition test in conditions 1, 2 and 3, but immediately after the first recognition test in condition 4, in which PAL testing took longer (*see* Table 1)

2. *Huppert-Piercy Recognition Test.* At the beginning of the day 1 testing session, subjects viewed 120 coloured slides of pictures taken from magazines on a Telex Caramate back-projector screen (*see* Huppert and Piercy, 1978*a*; Kopelman, 1985). They were asked to name one item from each slide, and to try to remember the slides. Ten minutes following the end of the slide presentation (during which they performed other tests), the subjects were given a recognition test, consisting of 40 of the original slides and 40 'distractors'. The subjects were told that they had seen some of the slides before and some not, and they were asked to say 'Yes' or 'No' according to whether they recognized each slide or not. Further recognition tests, each consisting of another 40 repeated and 40 distractor slides, were performed at a 2 h 40 min delay and at 48 h (*see* Table 1). In conditions 1, 2 and 3, the initial 120 slides were exposed at a rate of 0.5 s/slide. In condition 4, the exposure time was 'titrated' in an attempt to overcome any drug effect: 5 subjects viewed the slides for 1 s each and 5 subjects for 2 s each (mean = 1.5 s).

3. *Huppert-Piercy Recall Test.* This was performed on day 2, immediately following completion of the third recognition test. Subjects were requested to recall as many of the pictures they had seen 'today' as they could. They were encouraged to recall as many as possible, but were not given any specific cues or clues. The test was terminated after each subject had failed to recall any further pictures during a 30 s period. The subject was then requested to recall as many as possible of the pictures he/she had seen *only* on the 'previous' day. Scoring was in terms of the total number of (1) pictures correctly recalled from 'today'; (2) pictures correctly recalled from the 'previous' day; (3) temporal context errors, i.e., pictures recalled correctly but identified as having been shown on the wrong day.

4 *Paired-Associate Learning (PAL).* A list of 14 pairs of words was prepared, comprising 3 very easy (high associate) pairs (e.g., knife-fork) and 3 somewhat more difficult pairs (e.g., cup-plate) from Inglis' (1959) list, together with 8 'hard' (low associate) pairs (e.g., crush-dark) from the Wechsler Memory Scale (Wechsler, 1945). The experimenter read these word pairs to the subject in a randomized order (at a rate of 2 s/pair), after which the subject performed a 'distractor task' for 30 s, which was counting backwards by eights as fast as possible from an orally presented number (between 100 and 1000). At recall, the subject was given the first word of each pair in a random order and was required to remember its associate. Errors were not corrected, but there were 3 successive learning and recall trials in conditions 1, 2 and 3 (in which the order of the pairs was varied), and there were 5 successive trials in condition 4. In addition, a single 'recall' trial was administered at 48 h delay.

5. *Logical Memory Test.* At the start of the period of 'learning tests' on day 1 (Table 1), subjects were read the Anna Thompson story from the Wechsler Memory Scale (Wechsler, 1945). Recall was tested immediately following presentation, at 45 min and at 48 h delay.

6. *Word List Recall.* Four lists of 16 words were prepared (4 to 9 letters per word). Testing always began with a *primed recall* condition, in which subjects were not informed that recall would be assessed (*see* Shimamura, 1986). Each word from one of the lists was presented on a 'flash card' for 3 s and the subject was asked whether he/she regarded the word as 'pleasant' or 'unpleasant'. Following a 60 s distractor task, which was counting backwards by nines as rapidly as possible from a presented number (between 100 and 1000), the subject was shown the cue cards for that list (each consisting of the first 3 letters from one of the words) in randomized order, and he/she was asked to give 'the first English word beginning with those letters which comes into your head' (Graf *et al.*, 1984). There followed a *baseline* condition in which cue cards from another list were presented, without first displaying the words, to see how many words from the second list the subject would

give by chance to the same instruction. After this, there were *free recall* and *cued recall* conditions, whose order was varied between subjects. In both conditions, a list of words was presented with the same instruction and distractor task as in the 'primed recall' condition. During 'free recall' of the word list, the cue cards were not presented, whereas in 'cued recall' subjects were required to 'think of a word from the cards which began with the same three letters' (Graf *et al.*, 1984). At 48 h delay, subjects were presented with the cue cards from whichever list had been employed in the 'free recall' condition on day 1, and *delayed cued recall* was assessed. The word lists were balanced for word frequency (F(3,60) = 0.05), and the number of alternative words of comparable frequency (1-49 per million) beginning with the same initial 3 letters (Thorndike and Lorge, 1944). The list used for each recall condition was varied systematically between subjects.

7. *Modified Object Learning Test.* Cards 3 and 4 from Form A of the Kendrick Object Learning Test (Kendrick *et al.*, 1979) were presented to the subject for 60 s and 75 s, respectively, and recall of the pictured items was assessed. A modification of the usual procedure was that a 30 s delay was introduced between the end of card presentation and the start of recall. During this delay, subjects performed a 'distractor task', which was repetition of the 8-choice RT task with trials presented as rapidly as possible.

8. *Skill Learning* (*'procedural memory'*). A mirror-reading task was employed, based on the procedures of Martone *et al.* (1984) and Cohen and Squire (1980). Low frequency words (1-2 per million) of 8-10 letters (with a high prevalence of the letters b, d, u, n, p, q and g) were selected from the Thorndike-Lorge list. The words were typed in groups of three ('triads') and presented back-to-front as slides on the Telex Caramate back-projector. Twenty slides were presented. The subjects were required to read aloud each triad as rapidly as possible. They were informed whether an attempt at a word was correct or not. Presentation of each slide automatically switched on a timer, which the experimenter switched off as soon as the third word had been read correctly. A 'ceiling' of 100 s was allowed for each triad, and 'errors' (failed attempts at a word) were recorded. At 48 h delay, 20 further slides were presented with the same instructions. These consisted of 10 slides 'repeated' from day 1, randomly interspersed among 10 'new' slides. Following this second test session, subjects were required to write down as many words as they could recall from either test session. Thirty-five subjects completed the test in this form. An initial 5 subjects had viewed triads of (high frequency) three-letter words, but this test was found too easy and the material was changed.

9. *Verbal Fluency* (*semantic retrieval*) *Test.* On day 2, subjects were asked to give as many words as they could in 1 min beginning with the letters F, A or S in that order. The names of people or places or alternative forms of a single word were not permitted (*see* Miller, 1984).

10. *Remote Memory Test.* Subjects were shown 30 pictures of famous news events which had at some time been prominent in newspapers, magazines or the television news. They were asked a 'recall' question about the event depicted in each picture, and were then given 5 'recognition' choices, each of which was plausible but only 1 of which was correct. A longer version of this test has been validated in amnesic, dementing and healthy subjects (Kopelman, 1988). Because of their relatively young age, the present subjects were given a shorter version consisting of items from the period 1955-1984, 10 pictures from each decade in random order.

11. *Distractor Variation Task.* This was a variant of the Brown-Peterson test (Experiment 1), in which the difficulty (or 'processing load') of the distractor task was manipulated (*see* Morris, 1986). The same test material and projector system was used as in the Brown-Peterson test, but a number of modifications were made to the procedure. (1) There were 3 conditions, each with a different distractor task: 'articulation' (saying 'the, the, the . . .' repetitively), counting backwards by twos, and counting backwards by sevens, as rapidly as possible. There were 2 'practice' trials and 8 'test' trials in each condition. (2) In the 'counting backwards' tasks, counting began at the visual cue 'COUNT' from an orally presented number between 100 and 1000 (which was equally often an odd or even number in the twos condition). (3) Recall was tested at 5 s and 20 s delays only, randomly distributed within each condition.

RESULTS

Table 2 shows that the 4 groups were well matched in terms of mean IQ (F(3,60) = 0.77) as well as mean age and sex ratio. Consistent with the first experiment, hyoscine produced very significant changes in visual near-point, relative to placebo, on both days of the experiment (F(1,38) = 8.97, $P < 0.005$; F = 21.83, $P < 0.001$, respectively). Although the change in near-point was greater in the 'blind' than the 'nonblind' drug group on both days (for no clearly apparent reason), these differences were not statistically significant. Also consistent with the earlier experiment, hyoscine administration on day 1 produced a significant increase in the simple/8-choice RT difference (F(1,38) = 5.67, $P < 0.025$), as well as a significant effect upon change in 'subjective arousal' on both days ($P < 0.001$).

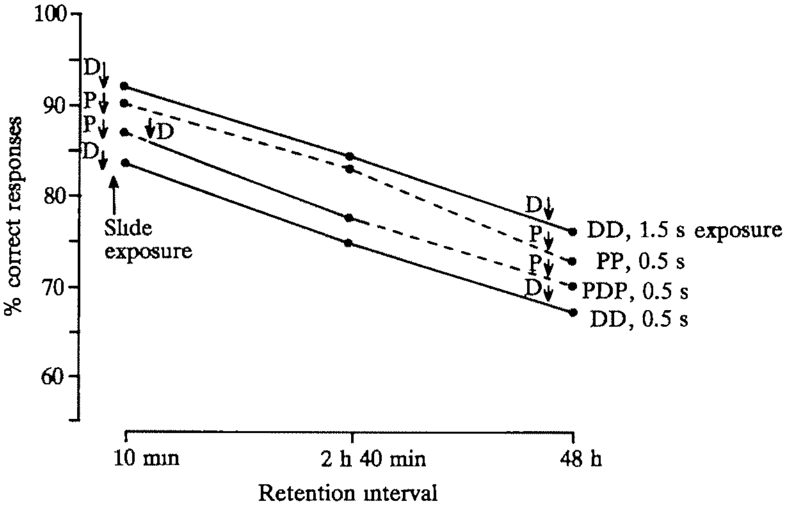

FIG. 4. Long-term forgetting test (Huppert-Piercy Recognition Test). Three groups viewed the slides for 0.5 s each, and the fourth group for a mean of 1.5 s per slide Group PP and both DD groups received an initial infusion at 0 min on day 1 and a 'shot' of placebo 60 min later (5 to 7 min after the end of recognition test 1). Group PDP received a placebo infusion at 0 min on day 1, an infusion of 0.4 mg hyoscine 1 h later, and placebo on day 2. Continuous line = drug/hyoscine; broken line = placebo.

Fig. 4 presents the results of the Huppert-Piercy Test in terms of correct recognition scores (true positive + true negative) at each delay interval. The combined placebo groups (PP+PDP) scored significantly higher at the 10 min recognition test than the hyoscine group, who had been given the same initial exposure time to the slides (DD, 0.5 s) (t = 2.19, $P < 0.025$, 1t; similarly, PP vs DD 0.5; t = 2.71, $P < 0.01$, 1t). This result is consistent with the hypothesis of a hyoscine-induced 'acquisition' deficit. (The higher level of performance of the PP than the PDP group at 10 min was not statistically significant.) The 'titration' procedure successfully overcame the drug effect, the 1.5 s/slide hyoscine group closely matching the first placebo group (0.5 s/slide) in terms of 10 min recognition score (t = 0.62, n.s.), and performing significantly better than the 0.5 s/slide hyoscine group (t = 2.62, $P < 0.01$, 1t).

The slopes of the curves indicate the forgetting rates of the 4 groups during the next 48 h. There was no significant (group × delay) interaction effect (F(6,72) = 0.25, n.s.), suggesting that forgetting rates were closely similar in the 4 groups. Individual comparisons of the PP group with the 3 other groups also gave no evidence of significant interaction effects. In particular, the PDP group did not show any significant 'dip' at the second test interval, which would have indicated either a retrograde or a state-dependent effect. Analysis in terms of the d prime statistic (controlling for differences in response bias) also did not produce any evidence of a difference in forgetting rates (overall interaction effect, F(6,72) = 1.28, n.s.).

TABLE 6 HUPPERT-PIERCY RECALL TEST

	'Today' correct responses (mean ±SD)	*'Previous' day correct responses (mean ±SD)*	*Temporal context errors*	
			Mean	*(% total correct responses)*
Placebo groups (PP+PDP)[1]	18.6 (±7.2)	8.6 (±6.5)	2.65	(9.7%)
Hyoscine groups (DD blind+nonblind)[2]	13.25 (±3.2)	5.55 (±4.45)	1.9	(10.1%)
t	3.03	1.73	1.07	
P	< 0.0025	<0.05 (1t)	n.s.	
[1] PP vs PDP: t =	0.80	0 20	0.66	
[2] DD blind vs nonblind: t =	1.67 (*P* > 0.10, 2t)	1.06	0.42	

Table 6 shows the results of the Huppert-Piercy Recall Test on day 2. The 2 drug groups and the 2 placebo groups scored similarly (*see* bottom 2 rows) and their results have been 'pooled'. It can be seen that hyoscine produced a marked reduction in the recall of slides which had been shown 'today' ($P < 0.0025$), and a somewhat lesser degree of impairment in the recall of slides which had been shown (following hyoscine administration) the previous day ($P < 0.05$). However, there was no significant difference between the 2 groups in the number or rate of temporal context errors made (i.e., slides correctly recalled but attributed to the wrong day).

Fig. 5 shows the pattern of results at paired-associated learning. The different levels of performance of the 2 drug groups (and the 2 placebo groups) on day 1 probably result from chance factors in subject recruitment. However, there was a consistent pattern whereby the 2 placebo groups showed a greater mean improvement in recall score between trials 1 and 3 (mean = +5.2, n = 20) than the 2 drug

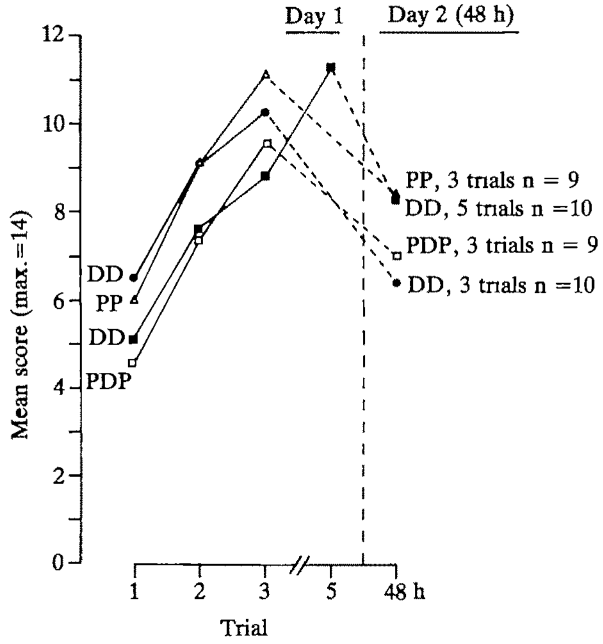

FIG. 5. Paired-Associated Learning. PP = placebo administered both days. DD = hyoscine both days. PDP = placebo, then PAL testing, then hyoscine on day 1, placebo on day 2. Data were missing from 2 placebo cases on day 2, and their results are not included in the plots.

groups (mean = +3.75, n = 20), a difference which was statistically significant (t = 2.24, $P < 0.025$, 1t), and which was mainly attributable to the learning of 'more difficult' and 'hard' paired-associates (*cf.* Winocur and Weiskrantz, 1976; Cutting, 1978). Thirty-eight subjects were retested on day 2, and their results are shown in fig. 5. It can be seen that the mean recall score (11.3±2.9) of the 'nonblind' hyoscine group after 5 learning trials closely matched that (11.1±2.7) of the first placebo group after 3 trials; at 48 h, the mean recall scores of these 2 groups remained closely matched (8.3 vs 8.4, respectively), indicating no difference in forgetting rate. Comparison of the mean 'forgetting score' (final score on day 1 minus day 2 score) of the combined hyoscine groups with that of the combined placebo groups also gave no evidence of a significant difference (t = 1.12, df 36, n.s.).

Fig. 6 plots word list recall, illustrating the mean number of words recalled in each condition. There were no significant differences between the mean scores of the 2 hyoscine groups ('blind' and 'nonblind') in any of the conditions, and their results are combined (group DD) in the figure. Group DP was tested following (blind) hyoscine administration on day 1, and (blind) placebo administration on day 2. The fig. shows that at 1 min delay, the hyoscine groups were severely impaired in the *free* recall of the word lists, relative to placebo group (t = 5.46,

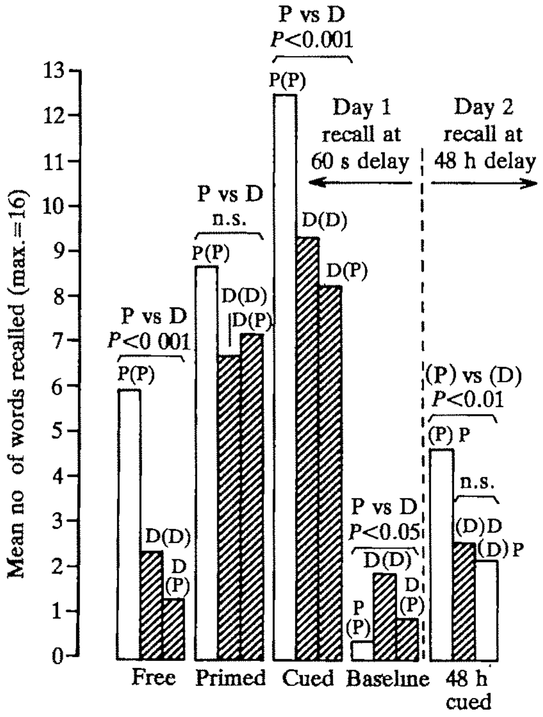

FIG. 6. Word list recall in free, primed, cued, baseline and delayed cued conditions. Open areas = placebo (P), hatched areas = hyoscine (D, 0.4 mg i.v.), where D(P) = hyoscine administered on day 1 and (D)P = placebo on day 2.

$P < 0.001$, 2t), whereas the small difference between the D(D) and D(P) groups was not significant (t = 1.74, n.s., 2t). At *primed* recall, the performance of the hyoscine groups (DD + DP) showed a greater improvement than did the placebo group (means = +4.87 vs +2.75), and the significant difference between the 2 groups disappeared (t = 1.74, n.s., 2t). By contrast, the improvement at *cued recall* was virtually the same in the 2 groups (means = +6.97 vs +6.55), and the significant difference between them remained (t = 4.03, $P < 0.001$). In the *baseline* condition, in which it was expected that the groups would respond by chance with virtually the same number of listed words, the combined 'drug' groups (DD + DP) scored significantly higher than the placebo group (t = −2.55, $P < 0.05$, 2t). However, this strengthens the earlier conclusions in that it suggests that the free recall performance of the hyoscine groups was more likely to have been influenced by guessing than the placebo group's performance.

At 48 h delay, the 'cued' recall performance of the placebo group remained significantly better than that of the groups (DD + DP) who had originally read the list after hyoscine administration (t = 3.24, $P < 0.005$, 2t). Moreover, the performances of the DD and DP groups were closely similar (t = 0.82, n.s.), indicating that there was no significant hyoscine vs placebo effect on retrieval. Moreover, hyoscine did not appear to have influenced the forgetting rate: comparison of cued recall score at 48 h with that on day 1 did not reveal any significant difference between the groups (PP vs DD+DP) in terms of either the absolute (day 1-day 2) or relative $\left(\frac{\text{day 2}}{\text{day 1}}\right)$ rates of forgetting (t = 1.29, 0.86, respectively).

Table 7 shows the results on the 2 remaining learning tests, the Wechsler Logical Memory and the modified Object Learning Test. On both tests, the hyoscine groups showed very significant impairment relative to the placebo group. Consistent with the earlier experiments, comparison of Logical Memory recall at 48 h with immediate recall failed to produce any evidence of a (group × delay) interaction effect (F(3,32) = 0.05 n.s.), indicating similar forgetting rates in the 4 groups.

TABLE 7 LOGICAL MEMORY AND OBJECT LEARNING RESULTS

	Wechsler Logical Memory (mean scores ±SD)			*Modified object learning test (mean scores ±SD)*
	Immediate	*45 min*	*48 h*	
PP	14.8 (±2.7)	12.1 (±2.5)	13.0 (±2.8)	26.9 (±6.25)
DD (blind)	11.4 (±3.1)	11.1 (±3.55)	9.7 (±2.7)	22.7 (±5.6)
DP	10.1 (±4.9)	7.9 (±4.25)	8.5 (±4.8)	18.2 (±5.2)
DD (nonblind)	10.6 (±2.1)	9.6 (±1.7)	8.8 (±1.6)	23.6 (±5.7)
t*	3.23	2.04	3.34	2.46
P(1t)	< 0.0025	< 0.025	< 0.0025	< 0.01

* PP vs combined D groups.

Fig. 7 shows the pattern of results on the skill learning (mirror-reading) task. The vertical axis shows the mean reading time for each triad in each 'block' of 5 trials. The horizontal axis indicates the 'blocks': 4 blocks of 'unrepeated' triads on day 1, 2 further 'unrepeated' blocks on day 2, and 2 blocks of 'repeated' triads (randomly interspersed among the unrepeated) on day 2. The mean rate of errors per subject (words failed or misread) was 5.28%; this was strongly correlated with mean reading time in the 'unrepeated' trials (r = 0.58, $P < 0.001$), but the mean number of errors did not differ significantly between the groups (F(3,31 = 0.98). Consequently, the further results will be reported in terms of reading speeds alone. A logarithmic transformation of the data was carried out to control for heterogeneity of variance between groups.

Fig. 7 shows that the initial reading speeds of the hyoscine groups tended to be slower than the placebo group, presumably a consequence of the effect on visual accommodation. This difference failed to reach statistical significance (F(3,31 = 2.23, n.s.), except when the placebo group was contrasted with the 'pooled' results

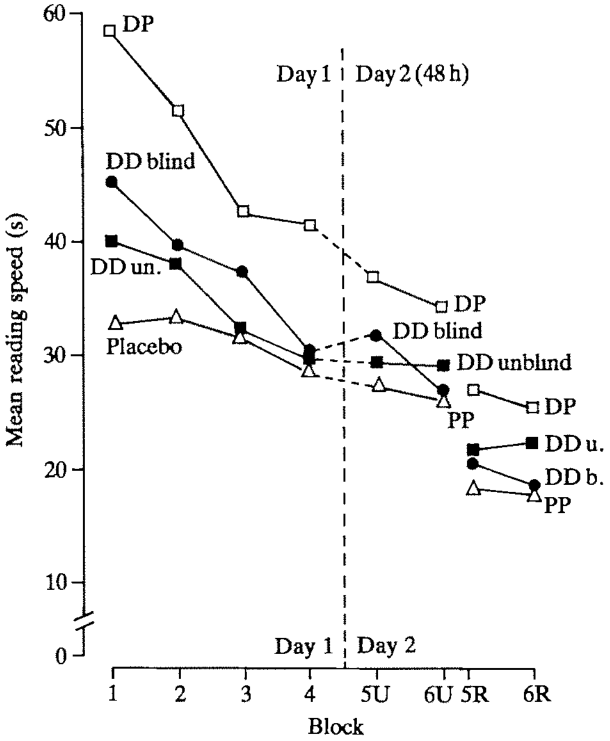

FIG. 7. Skill learning (procedural memory). PP = placebo administered both days. DD = hyoscine both days (tester 'blind' or 'nonblind'). DP hyoscine on day 1, placebo on day 2. 1 to 4 = blocks of (5) unrepeated trials on day 1. 5U, 6U = blocks of (5) unrepeated trials on day 2. 5R, 6R = blocks of (5) repeated trials on day 2, randomly interspersed among 5U and 6U, respectively

of the hyoscine groups (F(1,33 = 4.14, $P < 0.05$). As expected, there was a highly significant effect of delay interval during the course of the 6 'unrepeated' blocks, indicating improved reading speed with practice in all 4 groups (F(5,27) = 16.65, $P < 0.001$). On the other hand, there was no significant (group × delay) interaction effect (F(15,87) = 0.77), even when the placebo group was compared with the 'blind' hyoscine group in isolation (F = 0.84). This indicates that the groups did not differ in their rate of learning the mirror-reading task. At 48 h, the subjects showed markedly faster reading of the 'repeated' than 'unrepeated' triads (F(1,31) = 76.15, $P < 0.001$) but there was no significant group × repetition (F = 0.77) or group × repetition × delay (F = 0.03) interaction. This indicates that the drug groups were not differentially affected by repetition of the word triads. By contrast, in recalling as many of the words as possible at the end of the experiment, the placebo group (PP) recalled significantly more than the 'blind' hyoscine (DD) group (means = 9.5 vs 4.1, t = 2.25, $P < 0.025$, 1t), or all 3 drug groups (DD + DP) combined (t = 2.43, $P < 0.025$, 1t).

Table 8 shows the pattern of performance on the remote memory (news event)

TABLE 8 REMOTE AND SEMANTIC MEMORY

	News events test (1955–1984) *(mean scores ±SD)*		*Verbal fluency* *(semantic retrieval)* *(mean score ±SD)*
	Recall	*Recognition*	
Placebo	13.05 (±5.3)	21.20 (±5.25)	44.6 (±7.7)
Hyoscine 0.4 mg	15.15 (±4.9)	21.95 (±3 5)	43.6 (±12.5)
t	1.30	0.53	0.25
P	n.s	n.s.	n.s

and verbal fluency (semantic retrieval) tests. These tests were both performed on day 2 and, again, the results of the 2 hyoscine groups have been 'pooled' (n = 20), as have the scores of the 2 placebo groups (n = 20), because their results were closely similar on each of these tests. There was no significant difference between the placebo and hyoscine groups on the remote memory test, whether assessed by recall or recognition, nor on the verbal fluency test.

The final test was the Distractor Variation Task (fig. 8). Again, the results of the 2 placebo groups were closely similar, as were those of the 2 hyoscine groups, and the scores have been pooled to provide groups of 20 subjects each. Fig. 8 shows that, at the 5 s interval, there was a significant main effect of distractor task ($F(2,76) = 59.3$, $P < 0.001$) such that the 'counting backwards' tasks impaired recall, relative to 'articulation'. However, counting backwards by either twos or sevens had virtually identical effect ($F = 0.12$), presumably resulting from a 'speed/error trade-off'. A difference from the Brown-Peterson result in Experiment 1 was that the effect of hyoscine in the 2 'counting backwards' conditions was statistically significant ($F(1,38) = 14.74$, $P < 0.001$). However, there was

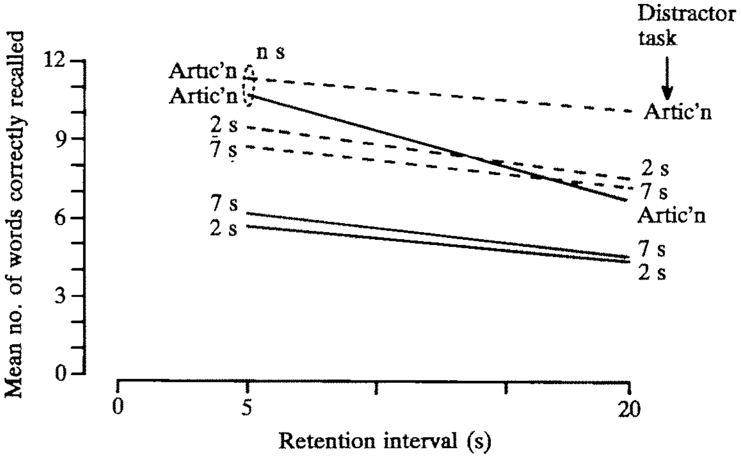

FIG. 8. Distractor Variation Task. Distraction = articulation (*see* text), or counting backwards by twos or sevens from an unpredictable number between 100 and 1000. Broken line = placebo, continuous line = 0 4 mg hyoscine.

TABLE 9 EXPERIMENT 2, DAY 1 TEST INTERCORRELATIONS (n = 40 EXCEPT WHERE STATED)

	Age	*IQ*	*Near-point*	*Arousal*	*RT difference*	*Logical memory immediate*	*Free recall*	*Primed recall*	*PAL difference*	*Object learning*	*H-P total correct*	*H-P d prime*
Age	1.00											
IQ	−0.24	1.00										
Near-point change	0.51***	−0.31	1.00									
Arousal change	0.12	−0.38*	−0.44**	1.00								
RT difference (n = 30)	−0.17	−0.17	0.23	0.33	1.00							
Logical memory Immediate (n = 36)	−0.33*	0.28	−0.55**	−0.33*	0.01	1.00						
Free recall	−0.32*	0.57***	−0.41**	−0.52***	−0.09	0.45**	1.00					
Primed recall	0.07	−0.01	−0.19	0.03	−0.06	0.31	0.10	1.00				
PAL difference (n = 30)	−0.43*	0.60***	−0.35	−0.25	−0.16	0.30	−0.56***	−0.37*	1.00			
Object learning	−0.30	0.32*	−0.32*	−0.43**	0.04	0.60***	0.65***	0.11	0.41*	1.00		
H-P 10 min total correct (n = 20)	−0.22	0.52*	−0.49*	−0.40	0.06	0.64**	0.54*	0.27	0.39	0.32	1.00	
H-P 10 min d prime (n = 20)	−0.08	0.43	−0.41	−0.38	0.01	0.59**	0.41	0.19	0.45*	0.15	0.92***	1.00

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ In view of the number of correlations in this matrix, significance values at the 0.05 level need to be interpreted with caution.

TABLE 10 EXPERIMENT 2, DAY 2 TEST INTERCORRELATIONS (n = 40 EXCEPT WHERE STATED)

	Age	*IQ*	*Near-point*	*Arousal*	*RT difference*	*Remote recall*	*Remote recognition*	*Verbal fluency*	*Distractor test*	*Word recall*	*H-P 'today'*	*H-P 'previous'*
Age	1.00											
IQ	−0.24	1.00										
Near-point change	0.33*	−0.22	1.00									
Arousal change	0.03	−0.17	0.38*	1.00								
RT difference	−0.11	−0.07	0.26	0.35*	1.00							
Remote memory recall	0.07	0.26	−0.15	−0.02	−0.06	1.00						
Remote memory recognition	0.24	0.27	−0.11	−0.02	−0.09	0.86***	1.00					
Verbal fluency (n = 26)	0.11	0.52**	−0.01	−0.15	−0.17	0.45*	0.43*	1.00				
Distractor test (total)	−0.35*	−0.52***	−0.64***	−0.45**	−0.08	0.18	0.23	0.22	1.00			
Word recall (mirror reading) (n = 35)	−0.49**	−0.48**	−0.30	−0.39*	0.10	−0.11	−0.14	0.16	0.59***	1.00		
H-P recall 'today'	−0.35*	0.27	−0.32*	−0.49**	−0.30	−0.25	−0.28	0.08	0.39*	0.63***	1.00	
H-P recall: 'previous'	−0.38*	0.35*	−0.22	−0.43**	−0.18	−0.07	−0.12	0.15	0.32*	0.50***	0.68***	1.00

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ In view of the number of correlations in this matrix, significance values at the 0.05 level need to be interpreted with caution

no significant (drug × delay) interaction effect (F = 0.26). It seems likely that the additional 'load' imposed by having to count backwards from a (verbally presented) unpredictable number between 100 and 1000 (instead of always from 100) may have contributed to the drug-induced impairment obtained in the present experiment. In the 'articulation' condition, there was no significant difference between the placebo and hyoscine groups at the 5 s delay (F(1,38) = 2.88, n.s.), consistent with the earlier Brown-Peterson result. More surprisingly, there was a highly significant interaction effect (F(1,38) = 14.67, $P < 0.001$), whereby the hyoscine group showed impaired performance at the 20 s delay.

Tables 9 and 10 show the test intercorrelations for tests performed on days 1 and 2 (except that RT difference scores from day 1 are also included in the day 2 matrix). The sample size is reduced for some of the tests, because groups in which the drug condition changed between performance of the tests or in which special conditions applied (e.g., prolonged exposure times in the Huppert-Piercy Test) are excluded from the calculations. There were satisfactory intercorrelations between many of the learning tests (e.g., between Logical Memory, free recall, PAL difference score, object learning, and Huppert-Piercy 10 min score on day 1). The change in visual near-point (the index of cholinergic blockade) correlated significantly with most of the main memory tests (logical memory, free recall, object learning, Huppert-Piercy 10 min and 'today' scores, and distractor test score). Change in 'subjective arousal' also correlated significantly with these tests, although there was only 14–19% of variance shared between change in near-point

TABLE 11 EFFECT OF COVARYING FOR NEAR-POINT AND AROUSAL CHANGE AND REACTION TIME DIFFERENCES (F values)

		Analysis of covariance				
Test	*ANOVA (One-way)*	*Change in near-point*	*Change in subjective arousal*	*RT difference*	*df*	*Groups*
RT difference	6.34**	0.41	1.42	—	2,26	P vs 0.2 vs 0.4
Nonverbal forgetting	5.68**	0.15	5.21*	3.17	2,26	P vs 0.2 vs 0.4
H-P 10 min recognition (total correct)	7.34*	4.17	3.19	5.71*	1,17	PP vs DD blind
H-P 10 min recognition (d prime)	6.51*	3.88	3.05	6.32*	1,17	PP vs DD blind
H-P recall. 'today'	9.20**	4.35*	1.11	5.99*	1,37	PP+P(D)P vs DD
PAL difference score	5.03*	1.48	1.49	4.33*	1,37	PP+P(D)P vs DD
Free recall: word lists	29.81**	19.72**	15.85**	30.38^{1}**	1,37	PP vs DD+(P)D(P)
Logical memory. immediate	10.45**	4.57*	6.05*	12.22^{1}**	1,33	PP vs DD+(P)D(P)
Word recall (mirror reading)	5.88*	3.87	2.83	6.48*	1,32	PP vs DD+(P)D(P)
Modified object learning	6.06*	2.92	1.57	7.13^{1}*	1,37	PP vs DD+(P)D(P)

**$P < 0.01$, *$P < 0.05$ [1] In the PDP group, these tests were measured in a different drug/placebo phase from 'RT difference', and a statistical adjustment on the basis of the other groups' results has been made to take account of this and change in arousal. By contrast, the RT difference score did not correlate significantly with any of the memory tests.

Table 11 examines this further with respect to the main tests which had produced significant differences in Experiments 1 and 2. It covaries the F values for change in visual near-point, change in subjective arousal, and RT difference score respectively. It can be seen that covarying for near-point and arousal change eliminates the significant differences on several of the tests, whereas covarying for RT difference has a negligible effect.

DISCUSSION

In summary, the present experiment has demonstrated impaired learning of (1) Verbal material, tested by recall (PAL, free recall of word lists, 'logical' memory); (2) Visuospatial material tested by recall (object learning test, Huppert-Piercy Recall Test); and (3) Visuospatial material tested by recognition (Huppert-Piercy Recognition Test). These results are broadly consistent with those obtained in earlier studies of cholinergic blockade (*see* Kopelman, 1986), but few of those studies employed such a wide range of tests or an index of central cholinergic blockade, and their methods often differed from those used in clinical studies of patients.

The rate of forgetting has been investigated in 2 principal ways. After matching for initial levels of learning on a verbal recall (PAL) and a pictorial recognition (Huppert-Piercy) test, it has been shown that the forgetting rate is unaffected by cholinergic blockade. Such a matching procedure has commonly been employed in clinical studies, but not previously in hyoscine studies. Secondly, administering hyoscine after initial learning did not affect the subsequent forgetting or retrieval of information (Huppert-Piercy Test, PAL), a result consistent with previous findings (e.g., Ghoneim and Mewaldt, 1975; Petersen, 1977). In addition, corroborative evidence that cholinergic blockade does not affect forgetting rates was obtained in the delayed, cued recall and logical memory tests.

Various aspects of memory were unaffected by cholinergic blockade. These included remote memory, whether assessed by recall or recognition, a result consistent with the finding that giving hyoscine after initial learning does not cause a retrograde impairment. Similarly, there was no loss of well-rehearsed semantic material, as measured on the verbal fluency test (*cf.* Beatty *et al.*, 1986). Implicit memory also appears to have been relatively unaffected by cholinergic blockade. The response to 'priming' was greater than that obtained in the placebo group, eliminating the significant difference between the 2 groups; but the significant difference in the response to 'cueing' remained (*cf.* the closely similar result in Korsakoff patients obtained by Graf *et al.*, 1984). Moreover, the rate of skill learning (procedural memory) did not differ significantly between the groups, and there was a trend for the hyoscine groups to show somewhat faster learning from a poorer level of initial performance. Finally, cholinergic blockade did not have a significant effect upon the rate of 'temporal context errors' in identifying on which of 2 days a slide had been seen before. This last finding is at first sight inconsistent with earlier reports by Caine *et al.* (1981) and Richardson *et al.* (1984), but those studies are not strictly comparable with the present, as they assessed the temporal discrimination of stimuli separated by only very short intervals (300 ms and 30 s, respectively).

The distractor variation result qualifies the earlier Brown-Peterson finding (Experiment 1). It suggests that if the distracting task is made more difficult (counting backwards from an unpredictable and unrepeated number), cholinergic blockade will produce a significant degree of impairment even at short retention intervals. The 'articulation' result at 5 s is consistent with the earlier Brown-Peterson finding, but the significant interaction with delay, producing impairment at 20 s, is more puzzling. Possibly the hyoscine groups found saying 'the, the, the . . .' for 20 s a more tedious (and thereby, distracting) task than counting backwards from 100 by threes, but this requires further investigation.

GENERAL DISCUSSION

Two issues remain. Can we attribute the deficits which cholinergic blockade produces on memory tests to impaired attention and/or subjective arousal? To what extent could cholinergic depletion account for the memory impairments of Alzheimer-type dementia and the alcoholic Korsakoff syndrome, if cholinergic blockade provides a 'model' of cholinergic depletion?

Of the 3 'objective' tests of attention employed in the present study, only 'RT difference' showed a significant degree of impairment following hyoscine administration. Covarying for 'RT difference' had a negligible effect on hyoscine-induced impairments in memory tests (Table 11). By contrast, cholinergic blockade produced a very significant effect upon 'subjective arousal', and covarying for this factor eliminated several of the significant memory effects, to an extent very similar to that produced by covarying for change in visual near-point (as a 'marker' of central cholinergic blockade). There are 3 possible interpretations of these findings: (1) cholinergic 'blockade' produces impaired 'arousal', and thereby an impairment of memory independent of any effect upon 'objective' attention; (2) it produces effects on both subjective arousal and 'objective' test performance, but the present 'attention' tests were not sensitive enough to pick this up; (3) the association between 'arousal' and memory test performance may be spurious, cholinergic 'blockade' producing independent effects on subjective and objective measures. This issue requires further investigation (*see* Wesnes *et al.*, 1988), but it can be noted that there was a relatively low degree of association between 'subjective arousal' change and visual near-point difference (as an index of 'objective' cholinergic blockade), their shared variance being only 14 to 19%. Moreover, the attention tests were selected for being broadly comparable with the memory tests in so far as both entailed a single channel of stimulus input operating over a relatively short duration of testing (*see* pp. 1105–1106), yet cholinergic blockade had pronounced effects upon the memory but not the attention tests. Taken together, these observations suggest that the effect of cholinergic blockade on memory performance may not necessarily be mediated by an action on either arousal or attention, and that it may produce partially independent effects upon these different aspects of cognition.

The present findings indicate that cholinergic depletion could only partially account for the deficits of primary or working memory seen in Alzheimer-type dementia. Performance at span tests and a conventional test of verbal short-term forgetting showed no or minimal impairment, similar to findings in some Korsakoff patients (Baddeley and Warrington, 1970; Kopelman, 1985) but very different from the severe impairment in Alzheimer patients (Corkin, 1982; Kopelman, 1985). On the other hand, a visuospatial short-term forgetting test, and varying the distractor conditions of the verbal test, did produce results similar to those obtained in Alzheimer studies (Morris, 1986; Sullivan *et al.*, 1986). It seems likely that cholinergic depletion does not mediate the impairment of Alzheimer patients at relatively 'undemanding' tests of primary or working memory, but it may contribute to their impairment where the 'information-processing' load of the test is greater, nonverbal tests being especially sensitive (*cf.* Sullivan *et al.*, 1986; Wesnes *et al.*, 1988).

An impairment of the anterograde component of secondary memory, implicating the recall and recognition of both verbal and visuospatial material, is of course characteristic both of Alzheimer-type dementia and the Korsakoff syndrome. The present results indicate that cholinergic blockade induces a similar (although less severe) pattern of impairment. In particular, it has been shown that, once learning has been accomplished, the rate of forgetting is unaffected, a result consistent with that obtained in Korsakoff and Alzheimer patients using an identical test (Huppert and Piercy, 1978*a*; Kopelman, 1985); moreover, this finding has been corroborated by the results of the PAL, delayed cued recall and logical memory tests. Also consistent with the findings of Korsakoff (and some Alzheimer) studies was the observation that 'implicit' memory appeared to be relatively intact: the response to 'priming' was relatively well preserved (*see* Warrington and Weiskrantz, 1970; Graf *et al.*, 1984) and skill learning (procedural memory) occurred at a normal rate (*see* Martone *et al.*, 1984).

On the other hand, various aspects of memory were unaffected by cholinergic blockade, despite being characteristically impaired in Alzheimer-type dementia or the Korsakoff syndrome. There is an extensive impairment of remote memory in both disorders, and the recall of temporal context is also characteristically impaired (Sanders and Warrington, 1971; Huppert and Piercy, 1978*b*; Kopelman, 1988; Sagar *et al.*, 1988). Similarly, long-established semantic knowledge, as measured by, for example, verbal fluency, is commonly affected in Alzheimer-type dementia (Miller, 1984; Ober *et al.*, 1986). These findings suggest that neurochemical or neuropathological lesions, other than cholinergic depletion, must mediate these deficits.

In conclusion, the present study indicates that cholinergic depletion could indeed produce an anterograde amnesia consistent with the pattern seen in the Korsakoff syndrome and many aspects of Alzheimer-type dementia. However, it seems unlikely that cholinergic depletion could account for the full range of deficits seen in the latter disorder, including the more passive aspects of primary memory and well-rehearsed components of semantic memory. The extensive retrograde impairment which occurs in both syndromes cannot be attributed to cholinergic depletion, and we must look elsewhere for a suitable explanation. Such findings have obvious implications for future studies of neurotransmitter function, as well as for clinical trials of 'replacement' therapies.

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IMPAIRED GRAMMAR WITH NORMAL FLUENCY AND PHONOLOGY

IMPLICATIONS FOR BROCA'S APHASIA

by STEPHEN E. NADEAU

(From the Geriatric Research, Education, and Clinical Center, Veterans Administration Medical Center, and the Department of Neurology, University of Florida College of Medicine, Gainesville, Florida, USA)

SUMMARY

Extensive testing of grammatical function, including assessment of spontaneous language, inflectional morphology, ability to produce grammatical structures, syntactic comprehension and grammatical judgement, was carried out in 2 patients with large dominant frontal lobe lesions, including but not confined to, the third frontal convolution. Both patients were fluent and had normal articulation and phonological production and neither was agrammatic, suggesting that even very large frontal lesions do not produce Broca's aphasia and that language cortex proper is confined to the postcentral perisylvian region. Both patients were impaired in the use of more complex syntactic structures and one, who in addition had severe generalized impairment in frontal lobe function, also had impaired judgement regarding the use and placement of functors. These data provide further support for the dissociability of syntactic and morphological aspects of grammar in aphasic patients and, together with other studies, link these functions with the frontal lobe and the postcentral perisylvian cortex, respectively. The sparing of grammatical judgement in 1 patient, despite a very extensive lesion, suggests that very large portions of the frontal lobe are involved in grammatical function. The nature of frontal lobe function in syntax appears to be congruent with the role of the frontal lobes in other aspects of behaviour.

INTRODUCTION

Ever since Paul Broca reported his famous patient and attributed his linguistic deficit to destruction of the dominant third frontal convolution, both the fundamental nature of Broca's aphasia and the lesion necessary and sufficient to produce it have been topics of controversy. Mohr *et al.* (1978) convincingly demonstrated that a lesion confined to the third frontal gyrus was not sufficient to produce Broca's aphasia and that this type of aphasia usually occurs with very large lesions, which initially produce global aphasia. Their findings have been replicated by Tonkonogy and Goodglass (1981). However, in neither of these studies did the investigators pursue detailed linguistic testing. While it can now be said that destruction of the third frontal convolution does not produce lasting linguistic impairment that is

Correspondence to: Dr Stephen E. Nadeau, GRECC (182), Veterans' Administration Medical Center, Gainesville, FL 32602, USA.

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readily evident at the bedside, we still do not know what linguistic function, if any, it does fulfill. Furthermore, the theory that the arcuate fasciculus serves to interface Wernicke's area with Broca's area has never been satisfactorily reconciled with the findings of Mohr *et al.* (1978).

At the same time that neurologists have debated these anatomical issues, neurolinguists have conducted extensive investigations into the nature of Broca's aphasia (Berndt and Caramazza, 1980; Kean, 1985). The outstanding feature of Broca's aphasia is agrammatism: the selective unavailability of closed class items (function words) with relative preservation of uninflected nouns, verbs and adjectives (Arbib *et al.*, 1982). Agrammatism may include impairment of syntax, which relates to the internal structure of sentences and the interrelationships among their component parts, and impairment of morphology, which relates to the internal structure of words and the interrelationships among words (Akmajian *et al.*, 1984). Grammatical aspects of morphology include the use of function words and the inflectional modification of words to indicate person, gender, and number, as well as derivational morphology, which will not be considered in this paper. Recent studies of aphasics have discovered dissociations between syntactic and morphological aspects of grammar (*see* Discussion), and lead to the question as to whether there is an anatomical counterpart to this functional dissociation.

This report describes 2 patients with large dominant frontal lobe lesions that included but considerably exceeded the third frontal convolution, one of whom had severe generalized impairment in frontal lobe function. Both patients were fluent and had normal phonological production; indeed, except for some mild anomia, neither had readily evident aphasia on bedside testing. The size and locus of their lesions and the remarkable superficial normality of their language led to a more detailed investigation of their grammar. This included a test of inflectional morphology (Experiment 1); a test of ability to produce grammatical structures, the Story Completion Test (Experiment 2); a test of ability to manipulate word order and function words, a sentence anagram task (Experiment 3); three tests of grammatical comprehension, one involving direct-indirect object relationships in the dative construction (Experiment 4), one involving locative prepositions (Experiment 5), and one involving word order and active and passive verbs (Experiment 5); and a metalinguistic test of ability to perceive syntactic relationships (Experiment 6). The results of these studies provide further evidence of a dissociation between syntactic and morphological aspects of grammar and suggest that the frontal lobes primarily subserve grammatical judgement and syntactic flexibility, linguistic processes that may be fundamentally similar to frontal lobe functions in other realms of behaviour.

CASE REPORTS

Case 1

J.B. is a 56-yr-old, eleventh-grade-educated, white female, trained as a nurse's aid, who enjoyed good health until 1976 when she developed visual difficulty, which ultimately led to the diagnosis of pituitary tumour. The tumour, an adenocarcinoma, was resected via a right frontal craniotomy, and she received postoperative radiotherapy. Immediately postoperatively, she was noted to have a right temporal hemianopia in her right eye, a superior temporal field cut in her left eye, bilateral optic atrophy and pituitary insufficiency requiring hormone replacement. Her family noted occasional problems with episodic memory but she readily resumed housekeeping responsibilities and was a capable cook. Two years later she married. She never returned to work because there was never a pressing need for her to do so and because her family was concerned that her memory impairment might cause employment difficulties. In December 1984, during a Christmas visit, her sister noted that J.B.'s memory was worse; she could no longer balance a cheque book, and she had become very demanding and easily agitated when things did not go her way During a subsequent visit in July 1985, a profound deterioration was noted in the upkeep of the home. J.B. sat around much of the day watching television and exhibited a complete lack of interest in doing anything else. She was irritated when her half-hearted efforts at housekeeping chores were criticized. She responded indiscriminately to television and mail order advertisements for magazine subscriptions and household articles, and piles of unread magazines were in evidence. By December 1985 she was no longer able to care for herself, although she insisted that she was quite capable of doing so. She had lost interest in all family members to the extent that it was thought she had forgotten they even existed. Previously of gentle disposition, she now was at times cruel and made jokes at the expense of others. Her days were spent doing nothing and she would often return to bed if left alone.

General physical examination was remarkable only for the presence of bilateral hip disease, which made most hip movements quite painful and severely limited walking. On neurological examination, the cranial nerves were intact except for the presence of bilateral optic atrophy and the previously mentioned visual field defects. Sensory and motor functions were completely intact and upper limb coordination was unimpaired. Her gait was hobbling and antalgic and required the use of a walking aid. The tendon reflexes were brisk and symmetric, and her plantar responses were extensor bilaterally.

J.B. was alert, cooperative, very friendly, somewhat childlike in her general demeanour and very good humoured, at times somewhat inappropriately She enjoyed attention and was an enthusiastic participant in all neuropsychological testing, although she required constant reassurance that she was making the correct response. She had always used her right hand or foot for all tasks except throwing or batting a ball and occasionally she would switch to her left hand while brushing her teeth. Her siblings and parents were all right handed She was able to remember none of 3 objects after 3 min of distraction. Immediately after hearing the first short story on the Russell–Wechsler logical memory test, her only correct recollection was that it involved a woman. Her response was a long, confabulatory account that seemed to include many events from her own life. After a 30 min delay she could not recall having heard a story. There was a severe disorder of remote episodic memory: all the major personal events of the past 10 years were reported as having occurred within the previous 6 months, their temporal order was confused, and their place of occurrence was often incorrect. She exhibited no right-left confusion. Praxis (facial and upper limb; transitive and intransitive) was completely intact. One-digit additions were easily executed but she had difficulty with any additions that required carrying numbers. There was no neglect or extinction. On the Thurstone word fluency test (F, A and S) she produced 3 words. She was able to name 6 animals in 1 min. Her performance on Luria sequential figure tasks was completely normal. These tasks consisted of copying long, randomly arranged sequences of circles, squares and triangles and continuing established recurring sequences of circles, plus-signs and triangles. On the first-edge-palm task, her movements were very irregular in timing and sequence and the same gesture was often repeated many times; when she simultaneously verbalized the sequence, she immediately performed correctly. On the Trailmaking test, part B, she seemed to comprehend the task easily but her performance consisted of connecting the numbers in correct order. On the Wisconsin Card Sorting Test she sorted according to number from the outset. After 20 such responses, she was carefully shown the source of her error and instructed on the next 2 samples as to how to perform correctly. She verbalized the way in which the old responses were incorrect and the new (assisted) responses were correct and

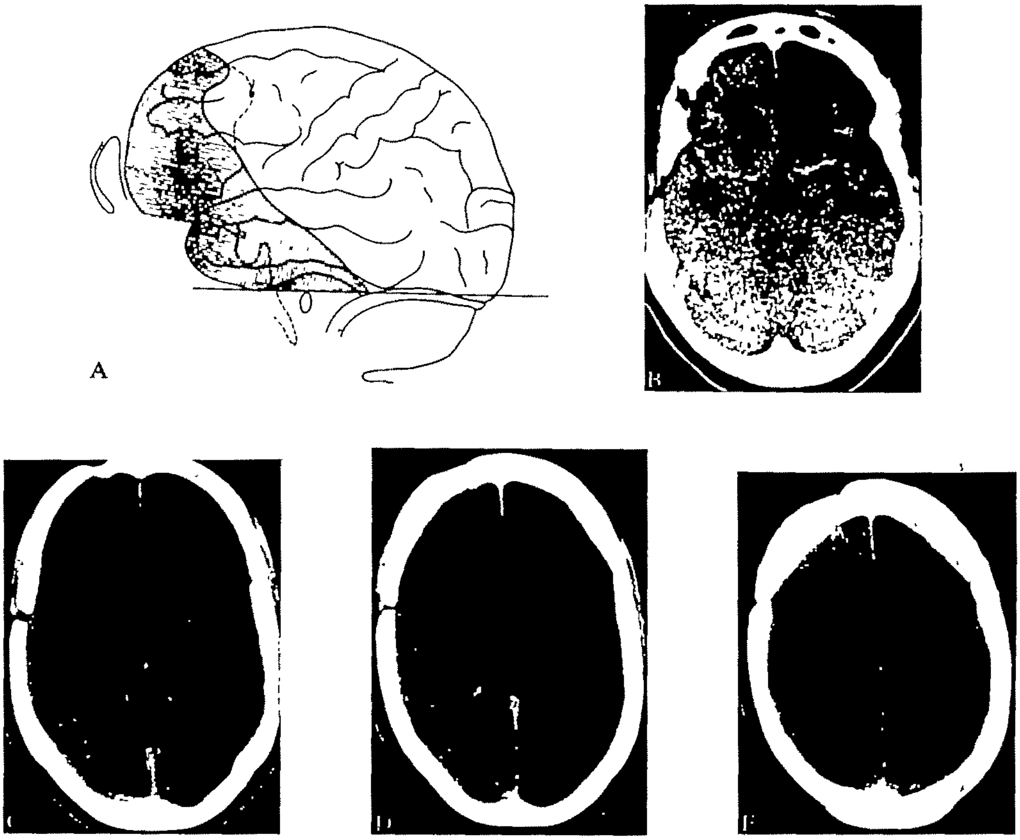


FIG. 1. CT scan from J B. obtained in November 1986. Four equally spaced levels are displayed (B–E), and the topography of the lesion has been mapped using the method of Mazzocchi and Vignolo (1979). The solid line and dark shading define the surface topography, and the dotted line and light shading define the subcortical lesion where it deviates significantly from the surface lesion

seemed very pleased when she made correct responses; however, she immediately reverted to sorting by number. In all testing sessions she exhibited a tendency to utilization behavior, constantly picking up objects and fiddling with them, at times using them in a manner implied by their design.

J.B.'s spontaneous language was completely fluent and while she produced simple sentences with little variety, it was not immediately apparent that she had a language disorder. Occasionally she suffered mild anomia and made verbal paraphasic errors. She achieved an aphasia quotient of 87 on the Western Aphasia Battery (WAB) and would be best classified as a mild anomic aphasic. Her performance was nearly normal except on sequential commands (score 70/80), object naming (score 48/60) and word fluency (score 6/20). On the test of reading comprehension, she missed 1 of 8 sentences.

A computerized tomographic (CT) scan obtained in December 1986 revealed an extensive region of diminished attenuation without mass effect in the left frontal lobe, which included the entire orbitofrontal cortex, the frontal pole and approximately 50% of the frontal convexity cortex, including nearly all of the inferior frontal gyrus but largely sparing the insula (fig 1). There was also a region of diminished attenuation in the deep frontal white matter on the right. These changes were interpreted as indicative of infarction due to radiation necrosis.

Case 2

W.G. is a 39-yr-old, eighth-grade-educated, white male who was employed as an iron worker until 1972 when he fell 25 ft and struck his head. The left eye was avulsed and he had extensive facial injuries but no skull fractures. He was in hospital for 1 month, after which he returned home with residua of a moderate right hemiparesis and focal and grand mal seizures, moderately well controlled with phenytoin. To this day, he remembers nothing of the hospitalization. Since the injury he has not been able to work because of his hemiparesis, and his main activity has consisted of socializing with friends. As before his accident, he continues to abuse alcohol regularly. He was recently convicted for complicity in postal money order fraud and currently lives in a halfway house completing his sentence. He and his first wife divorced in 1972, he married again in 1977 and divorced a second time 2 years ago. W.G. notes that his major problems since the injury have been impairment in recent memory, problems in expressing himself in that he has difficulty putting his ideas into words, and the loss of many premorbid skills. He was once a competent transmission mechanic but indicates that now he 'wouldn't know how to begin'. His mother denies any change in his personality. She notes that his recent memory function is adequate, that he is generally agreeable and cooperative, has normal impulse control and can be relied upon to complete satisfactorily jobs of which he is capable. She does complain that since the injury he talks faster, with poor articulation and is often very difficult to understand.

General physical examination was unremarkable. On neurological examination, the cranial nerves were intact except that he was completely blind in the left eye. The left optic nerve was atrophic and there was an associated afferent pupillary defect. There was a moderate spastic right hemiparesis mainly affecting distal musculature in the upper limb. He had essentially no fine motor movement in the right hand. Sensation was reduced to primary modalities and for graphaesthesia on the right. The tendon reflexes were hyperactive on the right, and the right plantar response was extensor.

W.G. was alert, cooperative and generally friendly but affect was rather flat. While quite agreeable to testing, he frequently claimed fatigue and asked to resume at a later time. He had always used his right hand and foot for all tasks until the traumatic hemiparesis forced him to make use of the left. He remembered 2 of 3 objects after several minutes of distraction. Immediately after hearing the first short story of the Russell–Wechsler logical memory test, he recalled all the major points while forgetting many details. After 30 min, he actually recalled slightly more than immediately following the test. Remote memory was intact. He exhibited no right–left confusion. Praxis (facial and upper limb; transitive and intransitive) was completely intact. He could perform only one-digit additions. There was no neglect or extinction. On the Thurstone word fluency test (F, A and S), he produced 25 words and he was able to name 16 animals in 1 min. His performance on the Luria sequential figures tasks was completely normal, and he was able to perform the fist-edge-palm task correctly without verbalization. On the Trailmaking test, part B, he performed rapidly and correctly, only occasionally missing a single number or letter in the sequence. On the Wisconsin Card Sorting Test (64 cards), he achieved 2 categories; of 31 errors, 21 were perseverative. There was no evidence of utilization behaviour.

W.G.'s spontaneous language was completely fluent and very rapid; sentence structure was simple, and variety of syntax was limited. Consonants were poorly articulated, making him difficult to understand. There was no evidence of anomia or paraphasic errors He achieved an aphasia quotient of 92 on the WAB; the few errors made were scattered through all parts of the test. Reading aloud and for comprehension was moderately impaired such that his score on the reading section of the WAB was 73/100. By history, his reading is no worse at present than before the accident. Most reading errors were made on the section assessing comprehension of sentences.

A CT scan obtained in February 1987 revealed a region of diminished attenuation without mass effect in the left frontal lobe, including the orbitofrontal cortex and the third frontal convolution, which was interpreted as residuum of a contusion (fig. 2) In addition, there was a region of diminished attenuation in the anterior cerebral/middle cerebral artery watershed region of the left frontal cortex that extended into the lenticulostriate watershed within the centrum semiovale. This was interpreted as evidence of infarction induced by traumatic carotid occlusion.

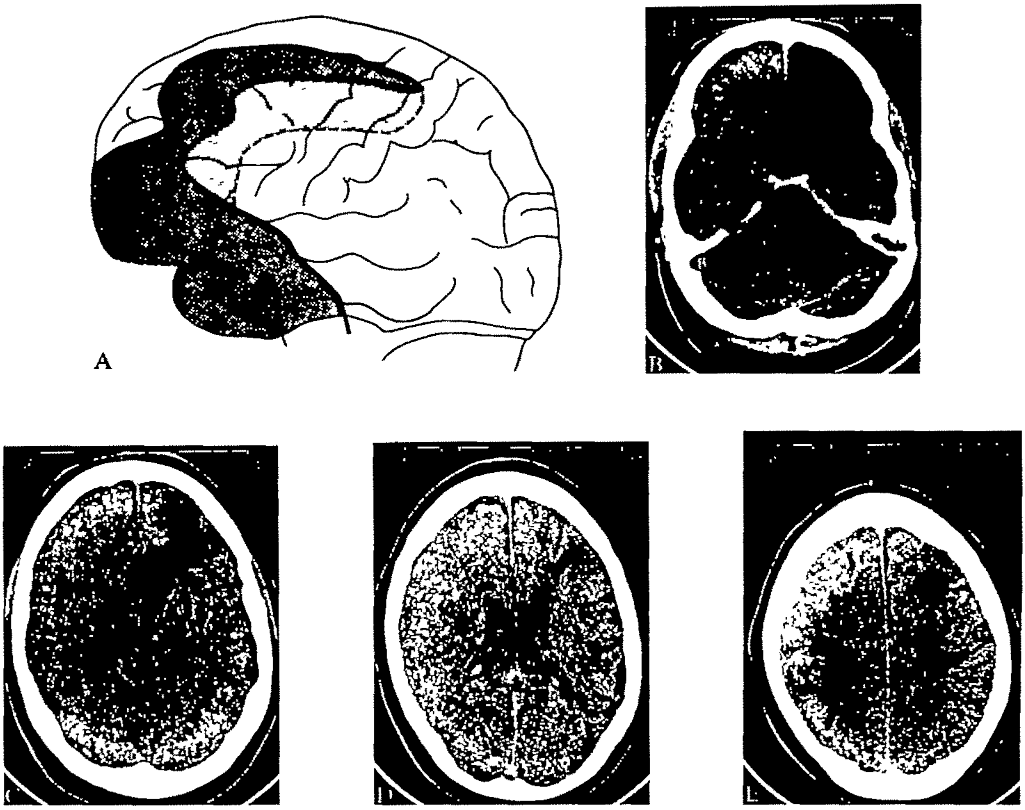


FIG. 2. CT scan from W.G. obtained in February, 1987. Four equally spaced levels are displayed (B–E), and the topography of the lesion has been mapped using the method of Mazzocchi and Vignolo (1979). The solid line and dark shading define the surface topography, and the dotted line and light shading define the subcortical lesion where it deviates significantly from the surface lesion.

RESULTS

In order to assess J.B.'s language function further, a series of experiments were conducted at her home over the course of 5 testing sessions of up to 3 h each over a period of 3 months. W.G. underwent the same testing over the course of 6 days of hospitalization following a series of focal seizures. Experiment 5 entailed the testing of control patients who were recruited from the neurology inpatient service at the Veterans Administration Medical Center, Jackson, Mississippi.

Experiment 1

Methods

Inflectional morphology was assessed using a form of the task developed by Goodglass and Berko (1960). This consists of a 60-item sentence completion test composed to include 6 opportunities for the use of each of the following 10 inflectional endings: plural [-z] or [-s]; plural [-əz]; past [-t] or [-d]; past [-əd]; present [-s] or [-z]; present [-əz]; possessive [-s] or [-z]; possessive [-əz]; comparative [-ər];

superlative [-əst]. An example of an item follows: 'The millionaire bought a new horse. He now has a whole stable full of .' Each sentence was read aloud by the author with slightly exaggerated phrasing and intonation.

Results and comment

J.B. responded correctly on 52 (87%) of the 60 items. Responses on 3 items were unscorable because she failed to recognize the item to which she was to add the inflectional morpheme. Three erroneous responses consisted of substitution of the superlative ending [-əst] for the comparative [-ər]. Two erroneous responses involved failure to add the complex possessive [-əz]. W.G. responded correctly on 47 (78%) of the 60 items. Responses on 7 items were unscorable because he failed to recognize the item to which the inflectional ending was to be added, or he provided an acceptable ending that did not make use of the desired inflection (3 responses). Two erroneous responses consisted of substitution of the superlative ending for the comparative; 4 involved failure to add the complex possessive. The 5 inflectional errors committed by J.B. and the 6 committed by W.G. compare with the mean of 22.8 errors made by 21 aphasic patients and the mean of 0.7 errors (range 0–3) made by 15 brain-injured nonaphasic controls tested by Goodglass and Berko (1960). The comparative ending was one of the easiest items for the aphasic patients of Goodglass and Berko (1960), and the difficulty J.B. and W.G. experienced may reflect educational and cultural factors. The only other item on which they made errors, the complex possessive, also proved to be one of the most difficult for the aphasic patients, who averaged 4 errors on this ending; it also accounted for 7 of the 11 errors made by the controls.

Experiment 2

Methods

Ability to produce grammatical structures was more systematically explored using the Story Completion Test (Goodglass *et al.*, 1972; Gleason *et al.*, 1975). The test consists of two variations of each of 14 different common English constructions (a total of 28 items). For each item, the examiner presents orally two or three sentences of a simple story to which the subject is expected to add a highly predictable final 'target' sentence or phrase. The following are 2 items with the correct response.

Imperative transitive. 'My little son eats lunch. He has not touched his milk. I want him to drink it. So I say: what?' Response: 'Drink your milk.'

Direct+indirect object. 'She owes her friend a dollar. She goes to see her friend. She takes out a dollar. What next?' Response: 'She gives her friend the dollar.'

The 14 different constructions included the following: imperative intransitive, imperative transitive, declarative intransitive, declarative transitive, direct+indirect object, yes–no question, WH question, future, embedded sentence, passive, comparative, cardinal number+noun, adjective+noun, and adjective+adjective+noun. The 28-item test was administered to both J.B. and W.B. on 2 different occasions and their answers were written down as they gave them.

Results and comment

Normal subjects produce the expected target response at least 90% of the time (Goodglass *et al.*, 1972). Two of J.B.'s responses were unscorable and she missed 17 others (31%). One of W.G.'s responses was unscorable and he missed 15 others (27%). Their performances were qualitatively as well as quantitatively comparable. Both missed half the direct + indirect object constructions. Two errors involved leaving out the direct or indirect object as in W.G.'s response 'She pays her' instead of 'She gives her friend the dollar', or incorporating the indirect object as the object of a sentence of address as in J.B.'s response 'Here's the dollar bill, my baby'. Two responses involved omission of the proper agent entirely, the indirect object becoming the new agent: to the story in which the desired response was 'I give the dog the bone', J.B. responded 'The dog takes the bone', and W.G. 'He eats it'.

J.B. missed 2 of 4 and W.G. 3 of 4 embedded sentence constructions. The erroneous responses invariably consisted of a simpler construction without an embedded sentence. For example, to the item 'The soldier's gun was dirty. The sergeant was annoyed. So he called the soldier over and told him he wanted: what?', which suggested the response 'He wanted him to clean his gun', the responses included 'He wanted his gun cleaned' (J.B.), 'He wanted to clean his gun up' (W.G.) and 'He wanted clean his gun' (W.G.).

J.B. missed all 4 passive constructions and W.G. 3 of 4. Five of the 7 errors consisted of semantically and grammatically correct sentences in the active voice. In 1 and possibly 2 of J.B.'s responses the verb became a descriptive adjective, the auxiliary was used as the copula, and the prepositional phrase specifying agency was omitted: to the story 'A little girl went too near the angry dog. What happened to her? She . . . What?' J.B. responded 'She became bit' and on another occasion 'She was bit'.

J.B. missed 1 and W.G. 3 comparative constructions. In every instance the erroneous response consisted of a noncomparative descriptive statement about one of the protagonists. For example, to the item 'Little Johnny couldn't reach the cookies. He wasn't tall enough. He called his sister and she reached the cookies for him. How come?' J.B. responded 'Because she was tall enough' and W.G. 'Cause he was too short'.

Both patients missed all 4 adjective + adjective + noun constructions, including only one of the two desired adjectives in their responses.

The overall performance of both patients on the Story Completion Test was markedly better than that of the Broca's aphasics studied by Goodglass *et al.* (1972) and Gleason *et al.* (1975). Much of the difference reflects their omission of few functors so that their responses were grammatical, if simplified: they had no difficulty with initial pronouns or subject-verb transitions and omitted no articles. Since a limited number of responses were elicited by administering the test only twice (compared with 5 times to each patient in the prior investigations), it might be argued that the errors committed might be serendipitous. Two observations argue against this. First, for each of the 5 constructions cited above (passive, direct + indirect object, embedded sentence, adjective + adjective + noun, and comparative, which accounted for 76% of J.B.'s errors and 100% of W.G.'s errors), an incorrect response occurred between 50 and 100% of the time. Secondly, these 5 constructions were generally those that proved most difficult for the Broca's aphasics tested in the two previous investigations, and the errors made by J.B. and W.G. were similar to those made by the Broca's aphasics. Only yes-no questions and future-tense responses, both of which entail using auxiliary verbs (e.g., '*Did* you brush your teeth?' 'He *will* work'), proved easy for J.B. and W.G. but very difficult for Broca's aphasics.

In summary, the Story Completion Test proved easy for J.B. and W.G. because of their normal fluency, phonology, and largely unimpaired inclusion of functors. Nevertheless, they performed substantially worse than the controls reported by Goodglass *et al.* (1972). While the normative data on this test are not optimal, it is worth noting that on many items the frequency and nature of the errors made by J.B. and W.G. were identical to those of previously tested Broca's aphasics. At the same time there were several items, most notably those requiring the use of auxiliary verbs, in which they performed normally, in marked contrast to Broca's aphasics.

Experiment 3

Methods

In order to assess syntactic processing capabilities further, a sentence anagram task was administered (von Stockert and Bader, 1976; Caramazza *et al.*, 1981). Each word from 16 sentences of various syntactic types was printed individually on a card. Each patient was presented with a set of cards for each sentence and was required to arrange them into a sentence. Either responses to the Story Completion Test (Goodglass *et al.*, 1972) or sentences used by Caramazza *et al.* (1981) in their sentence anagram task were employed. They included declarative intransitive, declarative transitive, direct + indirect object, yes-no question, WH question, future, embedded sentence and passive constructions.

Results and comment

J.B. had difficulty with 12 of the 16 sentences. With further effort she eventually arrived at the correct arrangement for 5 of these, and she required cuing on the remainder. Representative samples of her responses are given in the Appendix.

J.B. clearly had difficulty in using words that have primarily or exclusively grammatical value: articles were left out (sentences 2, 4) or preceded a verb (sentences 1, 5). Auxiliary verbs were commonly omitted or misplaced (sentences 3, 6, 7, 10), leading to major difficulty with future and passive constructions. The infinitive 'to' was commonly misused (sentences 8, 9). She also had problems in dealing with case. She failed to appreciate the possessive adjectival form of pronouns (sentences 2, 3, 7). The objective case was used in canonical subject position (sentence 8) or the nominative in objective position (sentences 9, 10). Even when the passive construction was finally recognized, the agent continued in the canonical subject position (sentence 10). Objects and agents were confused in embedded sentence constructions (sentences 8, 9), at times leading to absurd results. Occasionally, even subject-verb-object order was violated (sentence 7). J.B. eventually was able to achieve fairly good results, albeit with the help of some cuing, but her performance was quantitatively and qualitatively similar to that of the mild Broca's aphasic tested by Caramazza *et al.* (1981) and clearly inferior to that of the conduction and Wernicke's aphasics they tested. J.B.'s performance on the sentence anagram task paralleled her performance on the Story Completion Test, except that on the anagram task she even more closely resembled Broca's aphasics in that she experienced difficulty with auxiliary verbs and articles.

W.G. had difficulty with 8 of the 16 sentences. In 4 instances, he misread a grammatically incorrect arrangement to produce a grammatically correct verbal response (e.g., sentences 1 and 7, Appendix), and in a fifth instance (sentence 11) a similar misreading contributed greatly to his difficulty with the task. Of the 4 sentences on which he had difficulty with grammar, he eventually achieved the correct result without cuing on 3.

Two of W.G.'s errors involved placement of the auxiliary verb: 'did' in a yes–no question (sentence 4), and 'will' in a future construction (sentence 6). He had transient difficulty including all of the major lexical items in one of the embedded sentences (9) but all of his productions were grammatically correct, if at times awkward. One of the direct-indirect object constructions occasioned great difficulty in large part because the misreading of 'the' as 'they' gave him four nouns or pronouns to deal with. W.G. had none of J.B.'s problems with placement of articles, the infinitive 'to', case, or passive constructions, and with a single exception, he was able to use his grammatical skills in a productive fashion eventually to achieve a correct result.

Experiment 4

Methods

Comprehension of sentences employing the dative construction was tested using the picture selection task of Heilman and Scholes (1976). Each patient was presented one at a time with 20 sentences in which the meaning was dependent upon the indirect-direct object relationship, e.g., 'The man showed her baby the pictures'. Immediately before presentation of a sentence, each patient was shown 4 pictures on the same 8½ × 11 in sheet and asked to point to the picture that best corresponded to the sentence. Two pictures indicated 2 different indirect-direct object relationships. For the example given, there was a picture of a man showing pictures to a baby and there was a picture of a man showing a woman pictures of babies. The other 2 pictures indicated indirect-direct object relationships of completely different semantic content. If the subject pointed to the picture denoting the incorrect indirect-direct object relationship, this was scored as a syntactic error. If he or she pointed to a picture of different semantic content, this was scored as a lexical error. The 20 sentences were presented in auditory form on one occasion and in written form on a different occasion.

Results and comment

Neither patient made any lexical errors. J.B. made 6 syntactic errors (30%) during the auditory presentation and 5 errors (25%) during the visual presentation. W.G. made 3 syntactic errors (15%) during the auditory presentation and 9 (45%) during the visual presentation. This performance compares with a mean proportion of syntactic errors of 10% in controls, 42% in Broca's aphasics, 39% in conduction aphasics, and 57% in Wernicke's aphasics in the Heilman and Scholes (1976) investigation. A 50% error rate represents a chance level performance for the semantically competent patient who can immediately eliminate the semantic foils.

W.G.'s chance level performance with visual presentation appeared to reflect his poor reading.

Experiment 5

Methods

In order to study syntactic comprehension further, two tasks incorporating a picture identification format were used. In one, sentence comprehension depended on understanding the meaning of prepositional phrases. In the other, comprehension depended on correct interpretation of subject-verb-object relations as indicated by word order and voice markers. The subject and object in each sentence were either a square or a circle, thus preventing the use of pragmatic information to aid in sentence interpretation.

The first task (5A) was similar to task III of Schwartz *et al.* (1980). It consisted of 48 sentences, 24 describing the location of a square in relation to a circle alternated with 24 active-voice sentences in which a square and a circle were the protagonists. The 24 locative sentences incorporated 12 prepositions representing 6 polar contrasts: on-under, outside-inside, in front-in behind, above-below, to the right-to the left, in-around. Each preposition was used twice and was separated from its polar contrast by 5 different intervening locative sentences. Square and circle occurred equally often as the grammatical subject. Before being read the sentence, each patient was presented an 8 x 11 in card with 3 answer choices depicted by line drawings: one, the correct spatial relationship, a second, its polar contrast and the third, a lexical distractor incorporating a completely different, noncontrasting preposition. The position of the answers on the card was randomized. The 24 active-voice sentences included 2 sentences incorporating each of the following 12 verbs: touching, kissing, applauding, smiling, waving, phoning, drawing, kicking, chasing, following, shooting and pulling. In these sentences, the square and the circle were portrayed as stick figures acting out the various verbs. Again, square and circle occurred equally often as the grammatical subject. Before each sentence was read, each patient was presented with an 8 x 11 in card on which 2 answers were depicted, the desired answer and the reversed role distractor. The position of the answers was randomized. For all 48 sentences, the subject was required simply to point to his or her preferred choice.

The second task (5B) contained 24 active-voice sentences (as in 5A) and 24 corresponding sentences in the passive voice. Active and passive-voice sentences were randomly intermixed, and square and circle appeared equally often as the grammatical subject. The procedure was the same as in 5A, and the answer cards were the same as those used for the verb sentences in 5A, incorporating 2 choices, the correct answer and the reversed role distractor. Because this task had never been used previously, it was administered to J.B., W.G. and 5 educationally matched, nonbrain-damaged controls.

Results and comment

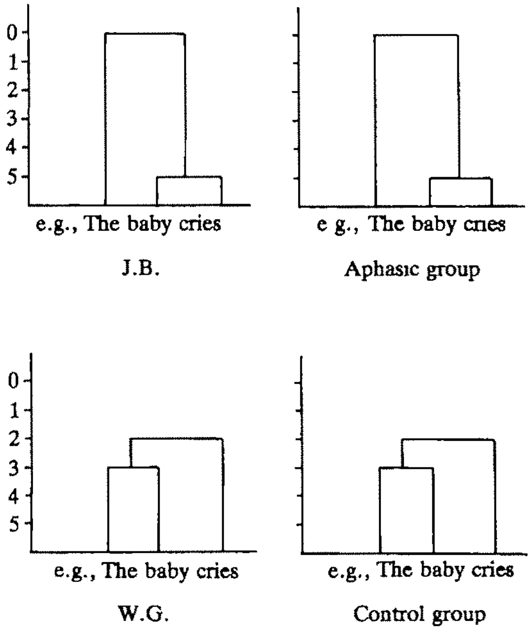
In experiment 5A, J.B. and W.G. were able to use either syntax or the principle that the first item was the agent and the second the object to respond correctly in 22 of 24 transitive verb sentences. The loss of pragmatic information was not a significant hindrance. On the prepositional sentences, J.B. missed 8 of 24 sentences, in 7 cases choosing the polar contrast, in 1 the lexical distractor. W.G. missed 4 of 24 sentences, in 3 instances choosing the polar contrast, in 1 the lexical distractor. Their error rates, 33% for J.B. and 16.7% for W.G., compare with 42% for the best of the Broca's aphasics studied by Schwartz *et al.* (1980).

In experiment 5B, neither semantic cues nor word order were reliable cues to sentence meaning. J.B. missed 5 (20.8%) of the constructions in the active voice and 3 (12.5%) in the passive voice. W.G. missed 4 (16.7%) in the active voice and 6 (25%) in the passive voice. The controls missed an average of 2 (8.3%) in the active voice and 5.6 (23.3%) in the passive voice. The scores for the control subjects were skewed by the performance of 1 patient who missed 15 (62.5%) of the passive sentences. The data suggest, somewhat surprisingly, that J.B. and W.G. may be scoring somewhat worse than expected on active-voice sentences. On the other hand, ability to comprehend the passive voice is evidently highly variable among patients with this educational background, and neither J.B.'s nor W.G.'s performance can be considered abnormal.

Experiment 6

Methods

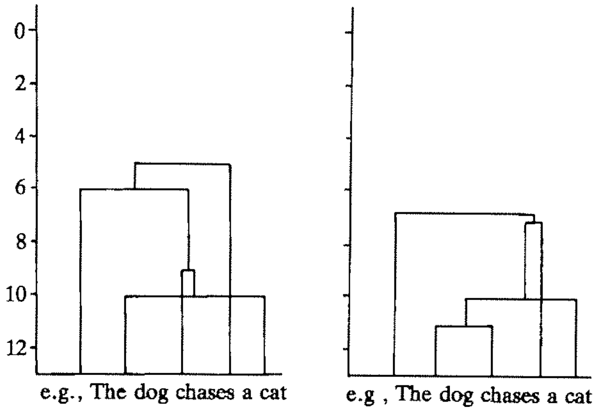
J.B.'s performance on the sentence anagram task (Experiment 3) suggested a problem in making grammatical judgements, notwithstanding her fluent, generally grammatical output. This was explored further using the metalinguistic task developed by Zurif *et al.* (1972). The task assesses the subject's intuitions regarding the degree of relatedness between words in a sentence. Because even normal subjects are apt to find this a very difficult task when sentence length is greater than 3 or 4 words, Zurif *et al.* (1972) instead presented their subjects with all possible 3-word combinations from each sentence and the subjects were asked to judge which 2 words were most closely related, based on the sentence. The ensuing data can be analysed using a hierarchical clustering scheme (*see* Martin, 1970, for details) if it can be assumed, as linguistic theory predicts, that the interword relationships within a sentence actually are organized in a hierarchy. The validity of this assumption can be tested by comparing the



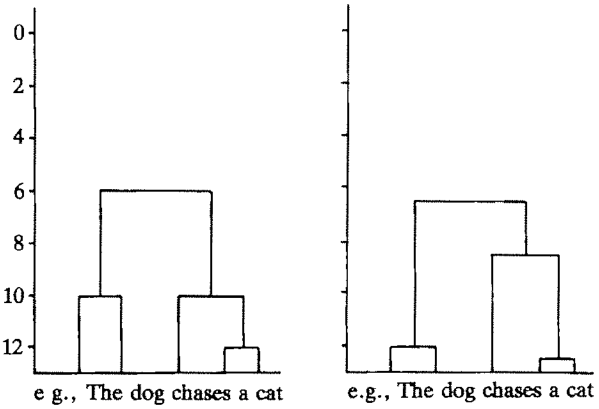
J.B. Aphasic group

W.G. Control group

FIG 3. Results of Experiment 6: declarative intransitive frame. Data from aphasic and control groups from Zurif *et al* (1972), with permission.



J B. Aphasic group



W.G. Control group

FIG. 4. Results of Experiment 6: declarative transitive frame. Data from aphasic and control groups from Zurif *et al.* (1972), with permission.

results of two methods of performing the clustering, termed maximal and minimal. If maximal and minimal solutions coincide perfectly, it can be concluded that the patient's judgements are influenced solely by the hierarchical scheme. The degree of disparity between solutions is a measure of the extent to which the judgements are influenced by nonhierarchical factors, such as the semantic content of individual sentences, biases introduced by the mode of task presentation (e.g., failure to take into account the effects of hemispatial neglect), distractions, or any other source of 'noise'. Zurif *et al.* (1972) found that in every aphasic and control patient they tested, the hierarchies indicated by maximal and minimal methods were topographically similar, although the precise degree of relatedness between any 2 words was often somewhat different.

The methodology of Zurif *et al.* (1972) was followed as closely as possible. Each patient was presented one at a time with 5 sentences representing each of 7 different constructions (35 sentences altogether, see figs 3-9). Sentences were printed on one line in capital letters on 5 × 7 in index cards, and each sentence was held in the patient's view thoughout the time he or she was judging word interrelationships. Each of every possible 3-word combination from the sentence was printed on a 3 × 5 in index

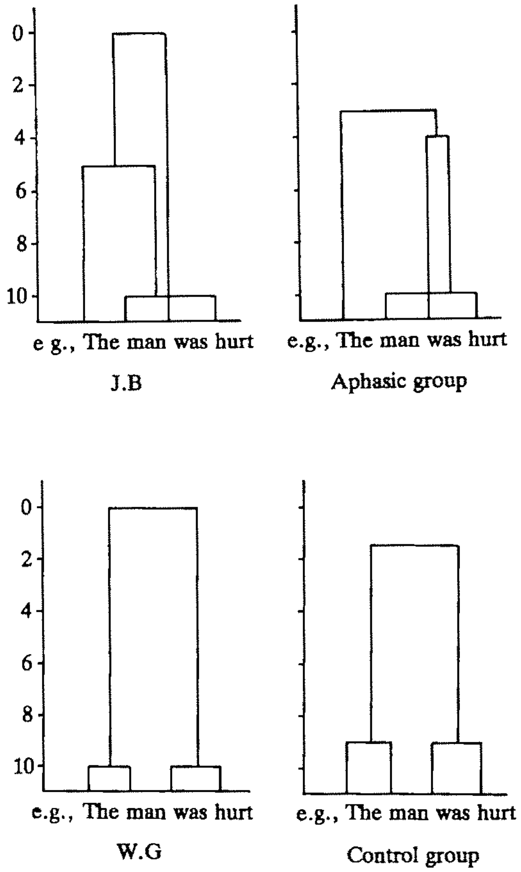
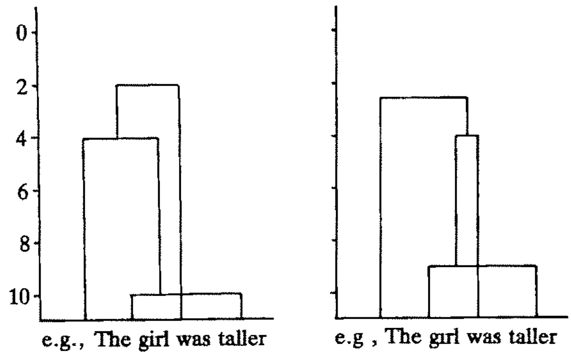


FIG. 5. Results of Experiment 6· passive frame. Data from aphasic and control groups from Zurif *et al.* (1972), with permission.

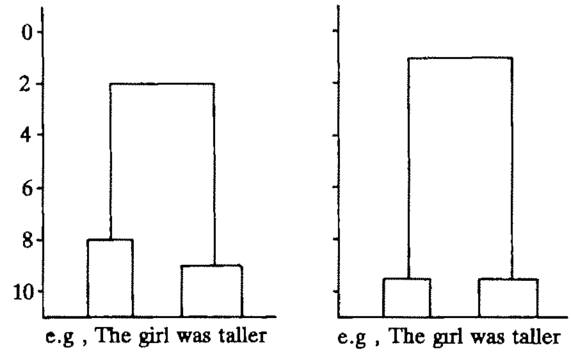
card such that the words formed the apices of an equilateral triangle with the word order from left to right reproducing the order of appearance of the words in the sentence. The order of presenting triads was randomized for each sentence. As each new sentence was presented and at other times during task performance deemed appropriate, the patient was instructed to 'pick the 2 of these 3 words that are most closely related to each other based upon this sentence.' The test was administered over two, approximately 1½ h sessions on two different days. To help assure the subject's understanding of the concept of sorting, he was administered a variety of sorting tasks, as described by Zurif *et al.* (1972), before beginning the test proper. In these tasks, the patient was presented with 3 two-dimensional geometric forms of various colours; in the first task, the subject had to select the 2 identically shaped forms and in the second, the 2 identically coloured forms. The third task was ambiguous in that of the 3 forms, 2 were of similar shape and 2 were of similar colour: the subject had to recognize the 2 sorting possibilities. Neither J.B. nor W.G. had difficulty with any of these.

Results and comment

J.B. and W.G. generally had little difficulty in performing this task. They typically read each sentence aloud and often verbalized 2 or all 3 of the possible ways the



J B. Aphasic group

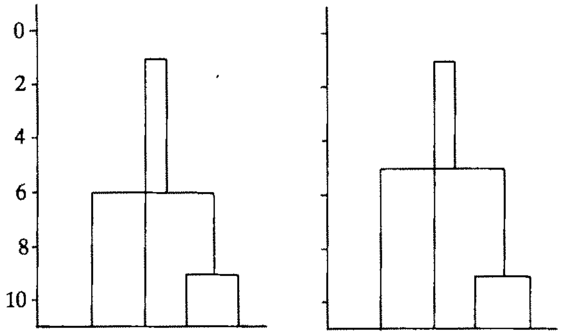


W.G. Control group

FIG. 6. Results of Experiment 6· comparative frame Data from aphasic and control groups from Zurif *et al.* (1972), with permission.

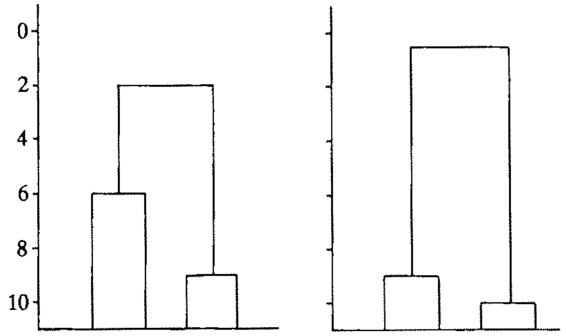
words on each card could be combined before selecting 1 of them. They frequently referred back to the target sentence in formulating their judgements. Hierarchical cluster analysis was performed for each patient for each of the 7 sentence types, and in all but one instance, maximal and minimal methods yielded topographically identical hierarchical structures, indicating that the patients' performances were consistent and not significantly influenced by semantic content or other factors. The one instance of topographic divergence—a minor one—involved W.G.'s assessment of the embedded sentence construction (fig. 9). The minimal solution placed the infinitive 'to' closer to 'speak quietly' while the maximal solution placed 'to' closer to 'Tom'. The minimal method outcomes are displayed in figs 3–9, along with analogous data from 3 patients with Broca's aphasia and 4 nonbrain-damaged controls tested by Zurif *et al.* (1972). In these figures, the higher the frequency of interword grouping (measured on the vertical axis), the higher the degree of relatedness of the 2 words.

The outstanding result of this analysis was that even though J.B. is highly fluent



e g , Where are my shoes? e.g., Where are my shoes?

J B. Aphasic group

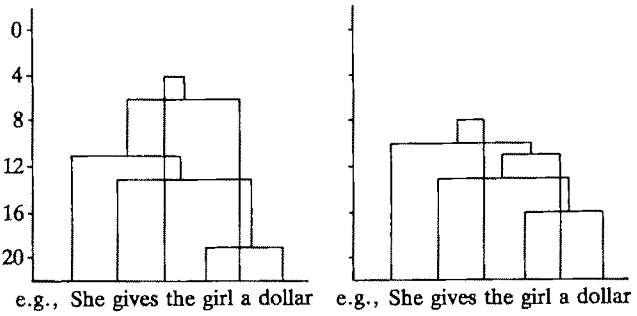


e.g , Where are my shoes? e.g , Where are my shoes?

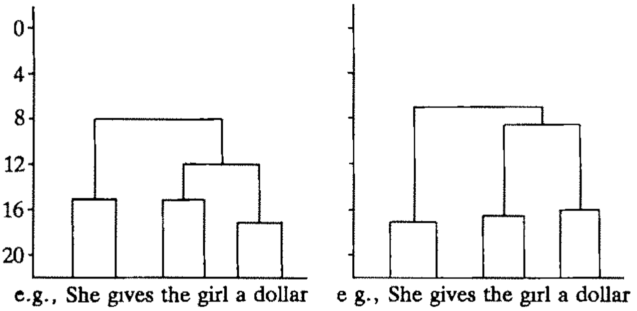
W.G Control group

FIG. 7 Results of Experiment 6: WH question frame. Data from aphasic and control groups from Zurif *et al.* (1972), with permission

and grammatical in casual conversation, her grammatical judgements closely resembled those of Broca's aphasics and were fundamentally different from those of controls. The underlying theme to her performance was that words that carried semantic value were singled out and clustered together, whereas words of purely grammatical function were relegated to a waste category. In this scheme, indefinite articles were almost never paired with their nouns or indeed anything else. On the other hand, for the possessive adjective (as in 'Where are *my* shoes?'), which is semantically marked, J.B. and the aphasics singled out a tightly organized noun phrase. Conjugations of the verb 'to be', used in a purely connective way as the copula in fig. 7, or as the passive voice marker in fig. 5, were not tightly related by J.B. or the aphasics to any other words in the sentence; controls reliably clustered the copula with the WH word and the passive voice marker with its verb. The clustering of words with semantic value also led J.B. and the aphasics to disregard subject-predicate boundaries (figs 4–6, 9). In marked contrast, W.G.'s grammatical



J B. Aphasic group



W.G. Control group

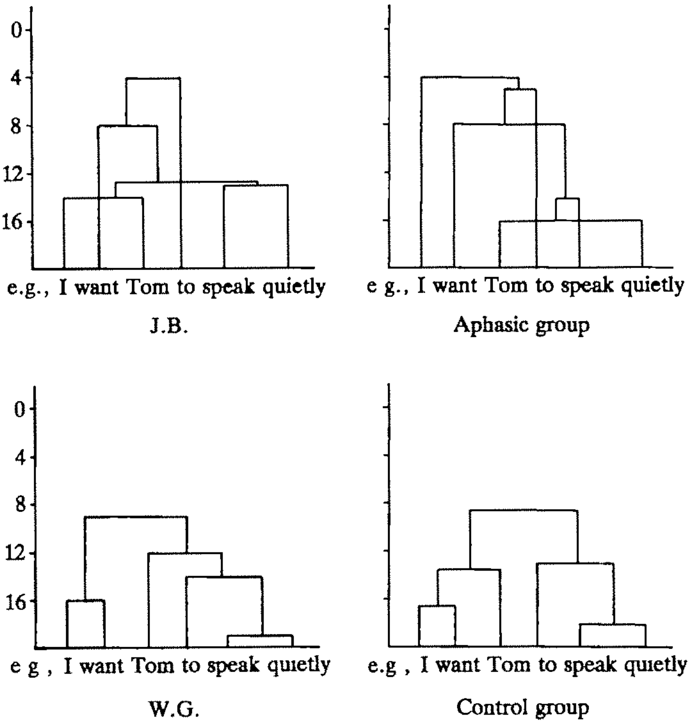
FIG. 8. Results of Experiment 6· direct + indirect object frame. Data from aphasic and control groups from Zurif *et al.* (1972), with permission.

judgement was virtually identical to that of controls tested by Zurif *et al.* (1972), his minimally impaired use of grammar, poor reading and limited education notwithstanding.

DISCUSSION

This report describes a 56-year-old woman who, 9 years following irradiation of a malignant pituitary tumour, experienced radiation necrosis that destroyed the orbital surface of her left frontal lobe together with the third frontal gyrus and anterior portions of the first and second frontal gyri. Her lesion conspicuously spared the motor strip, postcentral perisylvian regions, and the insula. Because the lesion resulted from radiation, it is likely to be confined to the region defined on the CT scan, which corresponds roughly to the radiation port, unlike primarily vascular lesions in which embolic fragments may disseminate widely through the various middle cerebral artery branches, many producing only laminar necrosis that is not apparent on the scan.

The second patient, W.G., is a 39-year-old white male who suffered a severe head



J.B. Aphasic group

W.G. Control group

FIG. 9. Results of Experiment 6: embedded sentence frame. Data from aphasic and control groups from Zurif *et al.* (1972), with permission.

injury 15 years ago with residua demonstrated by a CT scan of contusive destruction of the dominant third frontal convolution and orbitofrontal cortex, as well as watershed infarction of portions of the dominant high frontal convexity and centrum semiovale. His lesion, to the extent that it is demonstrable by CT scanning, also spared the perisylvian motor strip, postcentral perisylvian cortex, and the insula. On the other hand, because of the aetiology of the lesion, there may be substantial pathology not visible on the CT scan.

The anatomy of grammar

The spontaneous language of J.B. and W.G. was normal as far as it went, and was certainly not agrammatic. Nor was there any impairment in productive phonology. It gave the impression of simplification and lack of variability in syntax but no attempt was made to quantify this. Function words and inflectional morphology were normal in spontaneous language, and the latter was also very nearly normal on formal testing. On the other hand, when these patients were pressed to use more complex syntactic forms, as in the Story Completion Test, they both encountered considerable difficulty and, in particular, resisted using direct-indirect object constructions, embedded sentences, passive voice, and comparisons. On the sentence anagram test, both patients had difficulty in manipulating word order with respect to auxiliary verbs and embedded sentences. In addition, J.B. had difficulty on this task with the placement of function words such as articles and the infinitive 'to' and failed to respect case markers in word ordering. In J.B.'s case these difficulties were paralleled by a major disorder in grammatical judgement regarding function words. On comprehension tasks, only J.B. exhibited mild impairment, restricted to dative constructions and prepositional phrases.

In short, both patients exhibited impairment in grammar that was largely limited to syntax, and this impairment became manifest only when they were required to deal with more complex forms. On the other hand, although J.B.'s spontaneous use of grammatical morphology (both inflections and function words) was normal, she had lost the ability to judge the grammatical meaning of function words and had difficulty placing them when the main guide to their location was their grammatical meaning, as in the sentence anagram task. This impairment in turn might be expected to interfere with the creative use of function words in formulating more complex utterances.

J.B. and W.G. constitute examples of a dissociation between morphological and syntactical aspects of grammatical impairment that was first noted by Goodglass and Berko (1960) and has since been born out in a number of studies (Tissot *et al.*, 1973; Miceli *et al.*, 1983; Kolk *et al.*, 1985; reviewed by Goodglass and Menn, 1985). The patient reported by Kolk *et al.* (1985) is particularly noteworthy because she had a lesion confined to the motor strip and the parietal operculum; whereas her grammatical impairment was highly dissimilar and largely complementary to that of J.B., her spontaneous language was agrammatic. She had considerable difficulty in spontaneous speech and in formal testing with inflectional morphology, auxiliary verbs, the copula and the placement of articles, pronouns and prepositions but her grammatical judgement was completely normal and her spontaneous utterances were quite long. The nearly perfect double dissociation between the grammatical impairments of J.B. (largely syntactic) and the patient of Kolk *et al.* (1985) (largely morphological), the absence of any overlap in their lesions, and the presence of both types of grammatical impairment in Broca's aphasics who have lesions that subsume those of both these patients provide strong evidence for (1) the divisibility of grammatical function, (2) the localization of syntactic function to the frontal cortex and (3) the localization of a range of grammatical functions that subsumes such things as inflectional morphology, the use of the copula and auxiliary verbs and the placement of articles, pronouns and prepositions, to the postcentral perisylvian cortex. This proposition gains further support from other reports of dissociation between syntax and morphology, as noted above, as well as reports of impaired grammatical comprehension in conduction and Wernicke's aphasics who have lesions of the postcentral perisylvian cortex (Parisi and Pizzamiglio, 1970; Caramazza and Zurif, 1976; Heilman and Scholes, 1976; Samuels and Benson, 1979; Caramazza *et al.*, 1981; Kolk and Friederici, 1985). It also gains support from reports of similarities in the failure of Broca's and fluent aphasics to appreciate grammatical disagreement (Gardner *et al.*, 1975; Grossman and Haberman, 1982) and of abundant morphological errors among Wernicke's aphasics who speak morphologically rich languages such as German (Heeschen, 1985). Heeschen goes on to question the traditional distinction between agrammatism and paragrammatism: 'those syntactic phenomena that are omitted in agrammatic speech are present in paragrammatic speech but very often misconstructed and full of errors . . . (a)grammatics react to their syntactic trouble by avoiding any potential source of syntactic trouble in their spontaneous speech, whereas paragrammatics do not try to circumvent the danger points but run, as a German idiom says, into the open syntactic knife.'

The role of the frontal lobes in language

The findings presented here are in agreement with the previous reports by Mohr *et al.* (1978) and Tonkonogy and Goodglass (1981) that lesions of the dominant third frontal convolution do not produce Broca's aphasia. In fact, they indicate that very large frontal lesions extending considerably beyond the third frontal gyrus do not produce Broca's aphasia or, for that matter, any aphasia readily apparent at the bedside. Together, these data provide evidence against any role for prefrontal or premotor cortex in phonological production or speech articulation and, hence, against the very concept of a Broca's area. Instead, it appears that language cortex proper is confined to the postcentral perisylvian region, and that this cortex ultimately projects to the perisylvian motor strip, which mediates articulation (Lecours and Lhermitte, 1976). However, it must be admitted that the roles of the insula and posterior portions of the second frontal convolution remain in question.

A discussion of transcortical motor aphasia is beyond the scope of this paper. However, it may be noted that neither of our cases had this disorder, despite very large lesions immediately anterior to Broca's area, a location previously implicated in the genesis of this type of aphasia (Rubens 1976). On the other hand, J.B. and W.G. did exhibit one aspect of transcortical motor aphasia, which was a resistance to the use of adjective + adjective + noun constructions (Rubens, 1976).

J.B. and W.G. exhibited impairment in syntactic flexibility, and J.B., in addition, demonstrated a loss of judgement regarding grammatical morphology that would tend to interfere with the creative use of function words. These processes are quite consistent with the other functions subserved by the frontal lobes, in particular, planning behaviour that is precisely matched to the unique demands of a novel situation (a facility termed executive function by Stuss and Benson, 1986). For most behaviour, the novel situation is defined by circumstances external to the organism. In language production, the novel situation is defined by the particular network of concepts about which the subject is speaking and by the constraints imposed by what has already been said and how it has been said. Perfectly adequate English expression can be achieved using words of exclusively semantic value combined with the application of the subject-verb-object convention. Even tense can be expressed using adverbs of time without the need for tense markers. Grammar, on the other hand, provides the basis for the introduction of variety, efficiency and emphasis into expression: variety, through changes in word order and the use of clauses and embedded sentences; efficiency, through such things as elimination of the need to specify the agent by using the passive voice, the use of embedded sentences, direct–indirect object constructions, tense markers and inflectional morphology; and emphasis, through the use of cleft subjects and objects and demonstrative articles. Syntax also enables a general statement about relationships between semantic items to be tailored to a particular situation. For example, the general statement 'man hits girl' is transformed through syntax to the situation-specific statement 'it was this man that hit my girl'.

A yet-to-be-defined portion of the frontal cortex may influence the function of the postcentral cortex in the realm of grammatical function in a fashion analogous to the influence of the angular gyrus and its extensive connections with association cortices upon posterior perisylvian cortex in the realm of semantic function, as originally proposed by Geschwind (1965). In this conceptualization, J.B.'s aphasia reflects a problem with syntactic access just as anomic aphasia may reflect a problem with semantic access. Almost certainly, large portions of frontal cortex are involved in grammatical function; this is evidenced by the fact that W.G. had nearly normal grammatical judgement, despite CT-demonstrated lesions of the dominant third frontal convolution and convexity cortex. It is also born out by the fact that J.B.'s grammatical impairment occurred in the setting of severe generalized frontal lobe impairment.

J.B.'s impairment in grammatical judgement in which she consigned functors to a waste category, her difficulty with placement of functors in the sentence anagram task, and her mild impairment in syntactic comprehension most clearly demonstrated in Experiment 4 (dative constructions), also raise the possibility of a form of selective inattention to grammatical elements reminiscent of the frontal gnostic disorders described by Luria (1980).

In this revised model of frontal lobe participation in language function, the arcuate fasciculus cannot be a pathway by which linguistic data are carried from postcentral perisylvian cortex to Broca's area (*see* Geschwind, 1965), but rather one of the pathways by which the frontal cortex influences the activity of the postcentral perisylvian cortex. Case 1 of Miceli *et al.* (1983) may exemplify the effect of a lesion of the arcuate fasciculus. This patient suffered a stroke that produced a CT-demonstrated lesion involving the inferior fourth of the motor strip, extended slightly anteriorly into posterior portions of the pars opercularis (of the third frontal gyrus) and the second frontal gyrus and extended deep nearly to the putamen and lateral ventricle. This patient was initially mute, progressed through a phase of agrammatism and impaired articulation typical of Broca's aphasia, and ultimately demonstrated almost exclusively syntactic impairment with preserved morphology. Grammatical judgement, assessed by the method of Zurif *et al.* (1972), was normal after 14 months. According to our model, syntax was impaired because the arcuate lesion prevented the frontal lobe from exerting its influence on the postcentral perisylvian cortex in the realm of syntax. Grammatical judgement was spared because it was assessed using a metalinguistic task, which would not be expected to rely solely on the arcuate fasciculus, and because frontal cortex was intact. Morphological function ultimately recovered because only minor damage occurred in the postcentral perisylvian cortex, which was not demonstrated on the CT scan.

Syntax and concatenation

The data reported here and elsewhere that suggest dissociability of syntactic and morphological aspects of grammar in aphasia and which link these functions, respectively, with frontal and postcentral perisylvian cortex, provide some grounds for speculation about the fundamental nature of precentral and postcentral language function that may have important implications for research.

A large body of research has established that the frontal lobes play a major role in assembling set, structure and hypothesis from a collection of disparate pieces of data (Stuss and Benson, 1986). It comes as little surprise, then, that a patient such as J.B. with a large lesion of the dominant frontal lobe should exhibit impaired syntax and grammatical judgement. It also follows that research approaches founded in linguistic theory would have a reasonable likelihood of success in delineating the grammatical structure imposed by the frontal lobes. On the other hand, there is little reason to suspect, a priori, that the function of the postcentral perisylvian cortex is governed by a set of principles that might be organized into some kind of grammar. It is more likely that the function of this cortex is similar to that of the other nonfrontal cortex and reflects the representation in neural connectivity of an extremely large number of associations that have been formed in the course of language ontogenesis simply because they led to successful linguistic behaviour. In other words, it may make no more sense to talk of a grammar of the postcentral perisylvian cortex than it does to speak of a grammar of memory. Memory function appears to reflect the data screening functions of the cerebrum in conjunction with the fundamental properties of the neuronal systems that represent memory (Squire, 1982; Thompson, 1986). If the postcentral perisylvian cortex represents, in effect, a memory store for the vast data base that underlies morphology and phonology, then the effects of lesions of this cortex are likely to reflect the properties of neuronal rather than grammatical systems.

To elucidate further this dichotomy, I suggest that the terms syntax be applied to frontally-mediated grammatical functions and concatenation to grammatical functions mediated by the postcentral perisylvian cortex. The term syntax indicates that frontal grammatical function is mainly in the realm of syntax, but more importantly, and in the sense intended here, it conveys the concept that the dominant organizing principle is the manipulation of word order, functor selection and to a much lesser extent, morphological selection, to tailor linguistic output to the conceptual network and the constraints imposed by what has already been said. It seems likely that this manipulation is governed by a grammar. The term concatenation applies to grammatical morphology (inflections and function words) and productive phonology, but it conveys the concept that the dominant organizing principle is the sequential linkage of elements from the hierarchy of morphemic, phonemic and distinctive feature lexicons. This linkage may be constrained by the target open class words, by the next higher element in the hierarchy, by the influence of one element (or 'unit' in the terminology of Lecours and Lhermitte, 1969) on the choice of other elements in its neighbourhood, by the phonological prominence of the element and its position in the sentence (Goodglass *et al.*, 1967; Goodglass, 1976), and by other, as yet undefined, factors. In syntax, the selection and ordering of elements is sentence-based and reflects constraints imposed by the concept being articulated and presumably by a grammar. In concatenation, the selection and ordering of elements in the word or sentence reflect constraints imposed by the elements upon each other by virtue of rules of position, proximity and sequence incorporated in the neuronal substrate. Lecours and Lhermitte (1969), in their study of jargon aphasics, considered at some length the ways in which the processes governing the aphasic transformations of distinctive features, phonemes, joint phonemes and morphemes might correspond to the function of neural units.

It is very likely that the application of syntax and concatenation overlaps. Thus, J.B. and W.G. had grammatical but simplified spontaneous language and nearly normal comprehension; the patient of Kolk *et al.* (1985) had long utterances with good fluency but severe agrammatism because of a high incidence of morphemic omissions and nearly normal comprehension. When both syntax and concatenation are impaired, as in Broca's aphasia, the result is reduced fluency, agrammatism and impairment in syntactic comprehension.

The model developed here has a number of implications for research. First, research on syntax in aphasia may have a reasonable expectation of elucidating a grammar and might profitably be related to other aspects of frontal lobe function. Secondly, research on concatenation may be more profitable if it focuses on empirical observation that seeks to define the rules of position, proximity and sequence incorporated in the postcentral perisylvian cortex (e.g., Goodglass *et al.*, 1967; Lecours and Lhermitte, 1969; Goodglass, 1976). Thirdly, since concatenation incorporates aspects of both grammar and phonology, it may be more capable of lending order to observations of mis-selection of bound grammatical markers in languages such as Hebrew in which such markers cannot be omitted without the result being morphologically and phonologically ill-formed (Grodzinsky *et al.*, 1985). Fourthly, Goodglass and Menn (1985) have recently called for better use of controls in research on grammar. In this respect, Wernicke's and Broca's aphasia have long been viewed as polar opposites. Because the two share major features (Heeschen, 1985) reflecting impaired concatenation, it follows that anomic aphasia, stemming from a lesion of the angular gyrus that spares the perisylvian cortex (impaired semantic access, normal repetition, no literal paraphasias), is the appropriate control for Broca's aphasia. Given the uncertainties in localizing anomic aphasia, the recruitment of such controls will often require radiological confirmation. Finally, Zurif *et al.* (1988), in focusing on the on-line operating characteristics of components of language cortex, have provided evidence that the lexical access of an open class word in normal persons involves the exhaustive retrieval of all available interpretations and subsequently is limited to a single interpretation by the context in which the word appears. Agrammatic patients, in contrast, retrieve only the most frequent interpretation, regardless of context. This somewhat surprising lexical impairment of agrammatic patients might be explained best in terms of the well documented searching limitations of frontally-impaired subjects as revealed, for example, in their impaired performance on the Thurstone word fluency test (Hécaen and Ruel, 1981) and as revealed in part by the global remote memory impairment of Korsakoff's patients who have many cognitive deficits suggestive of frontal lobe dysfunction (Squire, 1982).

Conclusion

I propose that normal language production requires (1) semantic access, mediated by the angular gyrus and its extensive cascade of connections with association cortices (Geschwind, 1965); (2) concatenation, mediated by the postcentral perisylvian cortex; (3) syntactic function, mediated by the frontal lobes; and (4) articulation, mediated by the inferior motor strip (Lecours and Lhermitte, 1976; Tonkonogy and Goodglass, 1981). Lesions confined to the angular gyrus produce anomic aphasia without grammatical impairment. Lesions confined to the postcentral perisylvian cortex produce impairment in concatenation with attendant phonemic paraphasias and grammatical impairment (e.g., Kolk *et al.*, 1985), both of which may vary quantitatively and qualitatively depending on the locus of the lesion. Lesions confined to the frontal lobe may produce poverty of syntax and severe impairment in grammatical judgement but only mild overall grammatical impairment because concatenation is intact. Lesions involving both the angular gyrus and the postcentral perisylvian cortex often produce the uninhibited phonemic substitutions and paragrammatism with little content characteristic of Wernicke's aphasia because constraints of both semantics and concatenation have been lost. Lesions involving the frontal lobe and the postcentral perisylvian cortex, possibly including damage to the motor operculum, produce the telegraphic and profoundly agrammatic output of Broca's aphasia because neither syntactic function nor concatenation remain intact to support grammar or even the very presence of primarily grammatical elements. To the extent that there is a degree of overlap between the grammatical functions of the frontal lobes and the postcentral perisylvian cortex, as suggested by the performance of J.B. and the patient of Kolk *et al.* (1985), the grammatical dysfunction of the Broca's aphasic will be worse than the sum of its parts.

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APPENDIX

Results of Experiment 3

Successive responses to sentence anagram task

1. Target sentence: The dogs are chasing a cat.

J.B.:	W.G.:
The dogs are a chasing cat.	The dogs chasing
The dogs are chasing a cat.	The are dogs chasing a cat.
	(Read as 'There are dogs chasing a cat')

2. Target sentence: She gives her friend a dollar.

J.B.:	W.G.:
She gives dollar friend her.	She gives her friend a dollar.
She gives a friend her dollar.	

3. Target sentence: Did you brush your teeth?

J.B.:	W.G.:
Your teeth brush did you.	Did you brush your teeth.
Your teeth you brush did.	
(Cue: 'Start with did'.)	
Did your brush you teeth.	

4. Target sentence: Did you take the dog out?

J.B.:	W.G.:
Did you dog out take.	Take you out did.
Did you take the dog out.	Did you take out the dog.

5. Target sentence: When does the train leave?

J.B.:	W.G.:
When does the leave train.	When does the train leave.
(Cue: 'Read that for me')	
When does the train leave.	

6. Target sentence: She will begin work next Monday.

J.B.:	W.G.:
She begin next Monday.	Next Monday she begin will work
Will she begin next Monday.	Next Monday she will begin work
(Cue: 'She will . . .')	
She will begin work next Monday	

7. Target sentence: He will smoke his pipe

J.B.:	W.G.:
He pipe his smoke.	He will his smoke pipe.
He pipe will his smoke.	(Read as 'He will smoke his pipe')
(Cue: 'He will . . .')	
He will smoke his pipe.	

8. Target sentence: She wanted them to be quiet.

J.B.:	W.G.:
Them she to wanted quiet.	She wanted them to be quiet.
Them wanted she to quiet	
Them wanted she quiet to	
Them wanted she be quiet	
(Cue: 'She wanted . . .')	
She wanted them to be quiet.	

9. Target sentence: He wanted him to clean the gun.

J.B.:	W.G.:
The clean gun him he to.	He wanted.
The clean gun wanted him to he.	He wanted the gun.
(Cue: 'He wanted . . .')	He wanted the gun to clean.
He wanted the clean gun him to.	He wanted him the gun to clean.
(Cue: 'He wanted him . . .')	He wanted him to clean the gun.
He wanted him to clean the gun.	

10. Target sentence: She was bitten by the dog.

J.B.:	W.G.:
The dog bitten she was.	She was bitten by the dog.
The dog was bitten by she.	
The dog was bitten by.	
She was bitten by the dog.	

11. Target sentence: The boy gave it to the dog.

J.B.:	W.G.:
The boy gave it to the dog.	The gave boy it dog the.
	The to gave boy dog.
	The gave boy dog.
	Gave it to the boy
	The gave it to the boy dog.
	(Read as 'They gave it to the boy dog.')
	(Cue: 'The boy . . .')
	The boy gave it to the dog.

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WILSON'S DISEASE AND EPILEPSY

by T. R. DENING,[1] G. E. BERRIOS[1] *and* J. M. WALSHE[2]

(From the Departments of [1]Psychiatry and [2]Medicine, University of Cambridge Clinical School, Cambridge)

SUMMARY

The relationship between Wilson's disease and epilepsy is explored, both in the literature and in a series of 200 cases of Wilson's disease. Details of 44 literature and 14 personal cases of both disorders are presented. The prevalence on December 1, 1986 of epilepsy in the Cambridge series was 6.2%, ten times higher than that of epilepsy in the general population. Seizures in Wilson's disease occur at any stage of the disease, but often begin shortly after the start of treatment. Prognosis of seizures was comparable with the best quoted figures for idiopathic epilepsy: at 7 years 60% of cases had been seizure-free for at least 5 years, and 75% for at least 2 years. Possible mechanisms of seizures are discussed. Penicillamine-induced pyridoxine deficiency is probably not involved in more than a minority of cases. It is more likely that a direct effect of copper deposition is responsible for most of the seizures.

INTRODUCTION

The occurrence of epilepsy in patients with Wilson's disease (WD) is referred to in several review articles: 'Major epileptic seizures are occasionally seen and may occur at any stage of the illness' (Walshe, 1976); or 'In about 10% of cases the first indication of neurological disturbance may be either headache . . . or seizures. Seizures may be grand mal or partial complex. Seizures usually do (disappear with treatment) or, if persistent, are generally less frequent and severe' (Scheinberg and Sternlieb, 1984, p. 84).

In an attempt to clarify the relationship, we have reviewed the literature and analysed the occurrence and types of epilepsy seen in 200 patients with WD seen at Cambridge over the past 30 years. We have sought answers to the following questions. (1) Is there anything other than a chance relationship between epilepsy and WD? (2) What types of seizure are seen? (3) At what stage(s) may seizures be expected? (4) Do affected patients differ from others? (5) What is the prognosis of the two disorders? (6) What might be the causal mechanisms? (7) What is the effect of treatment?

LITERATURE REVIEW

The number of cases of WD in the published series and case reports studied was about 1000. Several series fail to mention seizures (e.g., Dobyns *et al.*, 1979; Lõssner

Correspondence to Dr T R Dening, Department of Psychiatry, Level 4, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

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et al., 1980; Giagheddu *et al.*, 1985; Starosta-Rubinstein *et al.*, 1987). Some authors mention the number of cases with seizures, but supply no further details (Bickel *et al.*, 1957; Okinaka *et al.*, 1961; Dastur *et al.*, 1968; Westmoreland *et al.*, 1974; Strickland and Leu, 1975; Xu *et al.*, 1981; Nevšímalová *et al.*, 1986; Przuntek and Hoffmann, 1987). A few cases may have had both disorders, but there is doubt regarding the diagnosis of WD (Anton, 1908; Greenfield, 1954), or of epilepsy (Franklin and Bauman, 1953; Jakab and Környey, 1948, second case; Streifler and Feldman, 1953) or both disorders (Glazebrook, 1945). Two cases definitely had both disorders but are reported elsewhere—the case of Yokoyama and Fischer (1914) was first published by Schütte (1913) and the case of Gibbs and Walshe (1966) appears as Case 11 in our own series below.

From the remaining literature, 44 cases with both WD and seizures, and adequate clinical description, have been identified. Another 30 or more cases probably had both disorders, but there was insufficient information for their inclusion. The 44 literature cases can be divided into 2 groups—those reported before the introduction of penicillamine (1954 or earlier) and those published since then.

In addition, several authors have examined the EEG findings in WD, and it is appropriate for these to be reviewed.

'Historical'

Table 1 summarizes information from 25 cases, published before the introduction of effective treatment for WD (Walshe, 1956). This material is therefore truly historical, and represents the 'natural' course of WD. The main criterion for inclusion in Table 1 is a typical history of WD; where corroborating evidence is available, it is listed. Cases with only a single seizure have been included for completeness.

In the first case of recognizable WD in the literature (Frerichs, 1861), a 10-yr-old boy, the terminal event was a series of convulsions. Wilson (1912, 1940) was doubtful as to whether epilepsy constituted a feature of his disorder, as none of his personal cases had seizures. Among the cases he collected from the literature was the family reported by Homén (1892, 1922) with at least 3, perhaps 5 affected members. One of these had tonic-clonic seizures; a younger brother had possibly had epileptic 'episodes of dizziness' in childhood. Another case (Võlsch, 1911) presented with a convulsion when aged 14 yrs, although she had no further seizures during the 3 remaining years of life. Thus despite Wilson's scepticism, 2 or perhaps 3 of the patients that he reported may have had epileptic manifestations.

Lhermitte (1912) published a case in the same month as Wilson's seminal article and, in referring to 'le syndrom de Wilson', may have been the originator of the term Wilson's disease. The 12-yr-old boy described developed generalized convulsions in the last few months.

The syndrome of 'pseudosclerosis' as described by Westphal (1883) and Strümpell (1898) is now considered to be one clinical presentation of WD, with tremor as the prominent manifestation. In contrast to Wilson's 'progressive lenticular degeneration', epilepsy was from the outset regarded as a key feature of this disorder. Both Westphal and Strümpell described patients who had seizures. Other reports of 'pseudosclerosis', although giving a typical history of WD, do not really satisfy other diagnostic criteria for WD, such as that of Leschtschenko (1925). In other cases of 'pseudosclerosis' (e.g., Syllaba, 1925; Alajouanine *et al.*, 1955), the demonstration of such features as corneal rings, cirrhosis and abnormal copper biochemistry make the diagnosis of WD incontrovertible.

The majority of cases in Table 1 are of 'neurological' WD, in keeping with Wilson's narrow conception of the disorder. The age at death is known in 20 of these cases, with the median survival after the development of neurological symptoms being about 5 yrs. Median survival after the emergence of seizures was 12 months, with 8 patients dying within a month or so. The appearance of seizures thus appeared to be an ominous sign, with status epilepticus especially lethal. It does not, however, appear that cases with epilepsy were in other respects more severely affected than those without seizures.

Recent literature

Since the introduction of chelating agents, the prognosis of WD has been transformed (Walshe, 1986). Many patients appear to have normal life expectancies. Table 2 reviews publications since 1955, a total of 19 cases of WD and seizures, taking into account the possible interaction between treatment both of the seizures and the metabolic disorder.

Opposing conclusions have been drawn from these reports, some claiming that epilepsy is uncommon (Mera, 1969) or that it is typical of ('pseudosclerotic') WD (Alajouanine *et al.*, 1955). It has been claimed that epilepsy is an early symptom or might even occur years before other symptoms (Chessa-Perle and Giagheddu, 1960; Smith and Mattson, 1967), whereas others believed it to be of late and sinister import (Alajouanine *et al.*, 1955; Capon *et al.*, 1973).

It has been thought that treatment of WD exacerbates seizures (Hollister *et al.*, 1960; Passouant *et al.*, 1969; Smith and Gallagher, 1970). Capon *et al.* (1973) suggested that response to anticonvulsants was poor. The occurrence of more than one seizure type in an individual patient has been described (Capon *et al.*, 1973). Alajouanine *et al.* (1955) believed that seizures resulted from cortical irritation, Passouant *et al.* (1969) incriminated disturbance of copper metabolism, while Smith and Gallagher (1970) attributed them to penicillamine-induced pyridoxine deficiency. We discuss this later in the light of our own observations.

The EEG in Wilson's disease

The first case report accompanied by EEG findings was published by Weatherly in 1941; the tracing showed episodic generalized and focal abnormalities. Other similar reports followed, although one article (Herz and Drew, 1950) described 5 cases, all with a normal EEG. Streifler and Feldman (1953) were the first to demonstrate an improvement in EEG with treatment.

TABLE 1 CASES WITH WILSON'S DISEASE AND EPILEPSY PUBLISHED BEFORE THE INTRODUCTION OF PENICILLAMINE (1861–1954)

Author	*Year*	*Country*	*Sex*	*Onset WD (yrs)*	*Onset ep. (yrs)*	*Death (yrs)*	*Presentation WD*	*Corroboration WD*	*Type of ep.*	*Other*
Frerichs	1861	Germany	M	9	10	10	Headache, lassitude, dysarthria	Cirrhosis, brain softening	Status	Died after 4 h
Westphal	1883	Germany	M	30	39	40	Oedema, pains in limbs	—	R focal motor + generalized	
Homén	1892	Finland	M	20	24	24	Listless, poor appetite, mental slowing	Cirrhosis, lenticular atrophy	Generalized	Died few wks later
Strümpell	1898	Germany	M	12	12	22	Seizure	—	Focal + generalized	No seizures from age 12–19
Völsch	1911	Germany	F	14	14	17	Seizure, dysarthria (6 mo. later)	Cirrhosis	Generalized	One seizure only
Hösslin, Alzheimer	1911	Germany	M	< 16	< 17	21	Poor work, tremor	Brain histology	Apparently generalized	No seizures in last 4 yrs
Lhermitte	1912	France	M	10	12	12 5	Dysarthria, dysphagia, dysgraphia	Cirrhosis, atrophy of putamen	Generalized	Died, 6 mo after ep
Schütte	1913	Germany	F	15	21	25	Anaemia, back pain	Cirrhosis, brain changes	Generalized × 1 R focal (yrs later)	Sister described by Steinmann, 1936
Hadfield	1923	UK	F	8–9	11	12	'Babyish', poor schoolwork	Cirrhosis, KF rings, atrophy of putamen	Generalized	Died in status
Leschtschenko	1925	Ukraine	F	12	12	?	Seizures, tremor	—	?Focal + 2° generalized	
Syllaba	1925	Czech.	F	14	14	30	Seizures	Cirrhosis, KF rings	?Generalized	3 seizures in total
Kryspin-Exner	1930	Austria	M	15	27	27	Tremor	Cirrhosis, brain histology	Generalized	Died 12 days after ep.
Lüthy	1931	Switz	F	18	54	61	Tremor	Cirrhosis, KF rings, PM, FH	Generalized tonic-clonic × 1 only	Sister had prob WD + focal seizures

Schneider	1932	Germany	M	?6	22	22	Personality change	Cirrhosis, atrophy of putamen	L focal + generalized	Died 6 mo. after ep.
van Gehuchten	1933	Belgium	F	9	9	?	Absences, poor schoolwork	—	Absences + occ generalized	
Steinmann	1936	Germany	F	23	25	25	Irreg. periods, weakness, wt loss	Cirrhosis, FH, atrophy of putamen	R focal motor	Sister of Schutte, 1913, died 8 days after ep
			F	15	15	?	Apathy, ataxia, personality ch.	FH	Generalized tonic-clonic	Living at age 17
Eicke	1941	Germany	F	childhood	3	24	Fits, poor schoolwork	FH, histology of putamen	Generalized tonic-clonic	
Jervis *et al.*	1942	USA	F	25	34	?	Psychosis	FH	Generalized tonic-clonic	Sister of following, alive at 50 but deteriorating
			M	38	48	48	Stammer, poor work	KF rings, FH, atrophy of putamen	Generalized tonic-clonic	Brother of above, one seizure only, died 2 mo. later
Messimy *et al*	1947	France	M	19	20	20	Invol. movts, dysarthria	Cirrhosis, KF rings, FH	L focal + generalized	Died 3 days after ep.
Jakab, Kornyey	1948	Hungary	F	17	18	18	Tremor, ataxia, dysphagia	Cirrhosis, KF rings, atrophy of putamen	1 R focal 2 L focal + generalized	Died 3 wks after ep 2nd case may have had ep.
Uzman, Denny-Brown	1948	USA	M	27	?	?	Tremor, affected sib.	KF rings, FH	Generalized	
Gysin, Cooke	1950	USA	M	18	23	23	Stiffness and deformity hands, psychosis	Cirrhosis, atrophy of putamen	Generalized ?1° focal	Died 1 wk after ep.
Franklin, Bauman	1953	USA	M	9	15	23	Ascites (9) tremor (15)	KF rings	Generalized	Case 3/11 ?2 others, with focal ep. but unclear

FH = family history; KF = Kaiser-Fleischer.

TABLE 2 CASES WITH WILSON'S DISEASE AND EPILEPSY SINCE

Author	*Year*	*Country*	*Sex*	*Onset WD (yrs)*	*Chelating agent (yrs)*	*Onset ep (yrs)*	*Presentation WD*	*Type of ep*	*EEG*
Alajouanine *et al*	1955	France	M	?5 ?20	27	27	Clumsiness (5) tremor, aggression (20)	Focal, gen + focal status	Slow waves L > R
Konovalov *et al*	1957	USSR	F	9	—	9	Seizures	Generalized	Frontotemp slow waves
Berard-Badier	1960	France	F	13	13 5	birth	Tremor, emotional lability	Generalized	Slow waves R > L
Chessa-Perle, Giaghoddu	1960	Italy (Sardinia)	F	?	—	6 mo	Tremor, ataxia	Generalized	L temp theta
Hollister *et al*	1960	USA	M	20	23	23	Blurred vision, stiff legs, dizziness	Generalized	L frontal focus + gen slowing
Heller, Kooi	1962	USA	M	18	20	22	?Tremor, ataxia	Generalized	R temp slowing
Poch, Martin	1966	Argentina	M	?	?	3 yrs later	Drooling, dysarthria	Focal + gen	R temp focus, gen slowing
Marín Hernandez *et al*	1967	Spain	F	21	—	23	Tremor, dysarthria	Generalized, myoclonic	R par theta spike + wave
			M	?	—	?	Tremor, myoclonus	Generalized	Centrenceph abn
Smith, Mattson	1967	USA	M	12	23	23	Lassitude, tremor	L focal + generalized	R front/temp focus
Hansotia *et al*	1969	UK	M	9	17	17	Not stated	Myoclonic, generalized	Multifocal + gen spikes
Mera	1969	Israel	F	?	9	10	Not stated	Status × 1	R post abn
Passouant *et al*	1969	France	M	12	14	14	Liver failure	Gen + focal	R + L front/temp foci
Smith, Gallagher	1970	USA	F	?24	25	26	Dystonic posture, tremor	Psychomotor status	R temp focus
Capon *et al*	1973	Belgium	M	12/13	15 6	15 5	Slow, dysarthria, dysgraphia	Gen + focal	R + L front + par foci
Ledic *et al*	1975	Yugoslavia	M	17	20	20	Clumsiness	L focal + status × 1	Diffuse theta
			M	?	34	33	Dysarthria, dysphagia	?Myoclonic	Diffuse abn
Brewer *et al*	1987	USA	M	19	22	22	Emotional	?Generalized	—
Lingam *et al*	1987	UK	M?	13	13	13	Fits, dysarthria, hallucinations	Generalized	—

The literature up to 1962 was surveyed by Heller and Kooi (1962), and this information, together with that from 4 other series appears in Table 3. These cases are probably not highly selected for patients with manifest seizures. Overall, 14/216 (6.5%) had seizures.

The EEG studies suggest the following conclusions. (1) EEG abnormalities are common, but most abnormalities are mild or moderate and nonspecific. (2) In general EEG changes mirror the severity of the clinical picture. (3) There is evidence that EEG changes improve with effective treatment of WD. (4) Actual seizures occur in a minority of cases with abnormal EEG records and tend to be associated with more severe EEG abnormalities. (5) EEG changes are common in heterozygotes: this and the nonspecificity of the changes render the technique of little use in screening for WD.

PATIENTS AND METHODS

A series of 200 patients with WD, all seen, investigated and treated by the same clinic, with up to 30 yrs follow-up, was studied with respect to psychiatric and neurological symptomatology. Cases

PENICILLAMINE (1955 TO DATE)

Anticonvulsants	*Response to anticonvulsants*	*Relationship and response of ep to WD therapy*	*Response of WD to WD therapy*	*Other*
Phenobarb, bromides, chloral	Partial	After/BAL seemed to cause seizures	Died aged 29	Related parents
Phenobarb	Partial	(Before)/—	—	Seizures preceded tremor by 6 yrs
Not stated	Poor	Before/possibly worse	Died aged 14	Diagnosis incompletely confirmed
?None	—	(Before)/—	None mentioned	
Phenytoin	Good	Before/—	Died 2 mo after first seizure	Received BAL + EDTA only L frontal lesion at PM
Not stated	—	After/—	Died 2 wks later	
Not stated	—	After/possibly worse	Partial improvement	Age of pt not given
Phenobarb	Partial	(Before)/—	—	2 sibs Authors uncertain of diagnosis of WD in both cases, but biochem data given suggests WD
		(Before)/—	—	
Phenytoin, phenobarb	'Ultimately' good	After/uncertain	Unknown	Penicillamine stopped due to bone marrow suppression
Not stated	—	After/good	?Improved	
Not stated	—	After/good	Unknown	Given pyridoxine with pen no seizures, but EEG remained abn
Phenobarb, phenacemide	Fewer attacks	After/good	Good	Seizures began when DTC substituted for pen Better when pen restarted
Phenobarb, phenytoin	Initially poor, good subsequently	After/good	Good	Status did not respond to phenobarb responded to iv pyridoxine
Not stated	Poor	Before/uncertain	Unknown	Seizures during first 8 mo of therapy, no further information
Phenobarb	Poor	Before/status after BAL	Died aged 20 in status	
Not stated	Poor	Before/good	Good	Authors refer to seizures as 'petit mal' but describe myoclonic ep
Phenytoin, phenobarb	?Good	After/good after 11 mo	Initially worse, later improved	Frontal lesions on MRI Changed from pen to zinc
None	—	Before/good	Good	

abn = abnormal(ity)

with seizures were identified retrospectively from medical notes. Only cases with more than one episode, where a good description (usually by a neurologist or experienced physician) was available, were included. EEG reports were available for most patients. This index sample was compared with nonepileptic controls (n = 182) on a range of variables using the Minitab statistical package.

To test whether the WD-epilepsy relationship differed from that expected by chance, reference data from the Rochester, Minnesota, study of epilepsy in the general population were used to compare rates of occurrence and types of seizure (Hauser and Kurland, 1975), and prognosis (Annegers *et al.*, 1979). The prevalence of epilepsy in our series was calculated for December 1, 1986

RESULTS

In total, 15 cases with WD and more than one epileptic seizure were identified (Table 4). One case (Case 14), the sister of Case 7, was not seen in Cambridge. Clinical information is available, but this case is not, strictly speaking, drawn from the Cambridge series; hence she has not been included in the statistical analysis. The unnumbered case had convulsions, but as they all occurred before the age of 3 yrs

TABLE 3 STUDIES OF EEG FINDINGS IN WILSON'S DISEASE

Author	*Year*	*Country*	*No. of cases*	*Abnormal EEG*	*Seizures*	*Other*
Konovalov *et al*	1957	USSR	28	21 (3 'severe')	1	Degree of EEG change correlated with clinical severity
Heller, Kooi	1962	USA	80 (literature)	43	3	In general EEG reflected clinical picture. ?Improvement with treatment
			7 (own)	3	1	
Hansotia *et al.*	1969	UK	28	15 (2 'severe') (13 'mild'/'mod')	1	No clear correlation with clin. and biochem. except in severely ill. EEG improved with treatment even in asymptomatic cases
Westmoreland *et al.*	1974	USA	33	18 (2 'severe') (16 'mild'/'mod')	7	In general EEG changes parallel clinical state and course
Nevšímalová *et al.*	1986	Czechoslovakia	40	31+ (1 'severe') (others 'mild'/'mod')	1	EEG changes common in heterozygotes too; EEG thus no use for screening
Totals			216	131+	14	

and were associated with fever, her inclusion as a case of epilepsy is open to question. She too has been omitted from the analysis. A further case from the series had a generalized seizure in hospital, but there was no evidence of attacks on other occasions. This patient has been regarded as having had a single seizure, and is not included in the Table. Thus 13 of the 200 patients in the series had both epilepsy and WD.

The 14 cases consist of 8 females and 6 males; 10 are still living. Eight were resident in the UK at the time they were seen; the rest were from abroad, all but one from Southern Europe. Mean age of onset of WD was 14.1 yrs, onset of epilepsy 16.6 yrs and start of treatment for WD 17.4 yrs. One patient (Case 6) had seizures before other symptoms led to the correct diagnosis, while another (Case 5), who had been diagnosed initially on family screening, developed seizures as the first manifestation of the disease. Seven patients presented with mainly neurological features, the others with a variety of symptoms, including haemolysis, growth retardation and liver disease. In 6 cases, epilepsy occurred before chelating agents were started, and in 8 cases treatment had already begun.

Four patients had focal motor epilepsy, and another had focal episodes of an unspecified nature. Eight others had generalized seizures, mainly tonic-clonic, although 2 cases had absence seizures only and 3 individuals had absence seizures as well as tonic-clonic attacks. Two patients survived an episode of generalized status epilepticus. The type of seizure in Case 14 was unspecified.

EEG reports were available for 13 of the 15 patients but unfortunately nearly all tracings had been destroyed. Ten were abnormal, including that of the case with febrile convulsions, and 5 of these showed a focal abnormality. Serial reports were available for 4 cases, all showing improvement with treatment.

Thirteen patients received anticonvulsants, 7 responding well (i.e., no further attacks). Four patients had a reduction in the number of seizures, and 2 were not improved by anticonvulsants. Treatment of WD ended seizures promptly in 8 patients, and after 3 yrs in 2 others. Three cases responded partially, and 1 (Case 9) continued to have more frequent seizures. The case with febrile convulsions had no further attacks after starting penicillamine. The response of the WD itself to treatment was more variable, but with the exception of the 2 earliest cases, most have improved.

A statistical comparison between epileptic and nonepileptic patients showed that epileptic cases displayed more incongruous behaviour (defined operationally as a dissociation between environmental cues and behaviour, including disinhibited, bizarre and reckless conduct) ($\chi^2 = 6.2$, 2 df, $P < 0.05$) and received more medication (a difference accounted for by the prescription of anticonvulsants) than nonepileptic cases ($t = 2.58$, $P < 0.03$). No other significant differences emerged.

The comparison of prevalence, classification and prognosis of seizures between the Cambridge WD series and the Rochester data is shown in Table 5.

TABLE 4. EPILEPSY IN THE CAMBRIDGE SERIES OF

Case	*Sex*	*Country*	*Birth year*	*Onset WD (yrs)*	*Penicillamine (yrs)*	*Onset ep (yrs)*	*Presentation WD*	*Type of ep*	*EEG*
1	F	UK	1946	12	13	13	Weakness, clumsy	Focal + gen	Nil specific
2	F	UK	1946	6	14 5	14	Haemolysis, jaundice	R focal motor	Widespread theta
3	F	UK	1957	11	18	20	Dysarthria, clumsy	Gen (absence)	Minor abn only
4	M	UK	1960	18	18	19	Tremor, dysarthria	Gen (absence)	Gen slow waves
5	M	UK	1966	13	13 5	13 3	Asympt (affected sib)	Generalized	Sl paroxysmal abn
6	M	UK	1961	19	19	8 12	Jaundice, oedema, epistaxes	Gen (absence) tonic-clonic	—
7	F	UK	1950	29 25	29 3	29 5	Stiffness, poor gait	R focal, gen + status × 1	L post focus
8	M	UK	1938	12	21	18	Retarded growth, bone disease	R focal + gen	L frontal abn
9	F	Italy	1955	6	11	?11 ?15	Hepatosplenomegaly, dysarthria aged 9	Generalized	Gen abn R > L
10	M	Italy	1956	7	13	14	Hepatitis, dysgraphia aged 12	Gen × 2, partial × 1	Paroxysmal bilat R > L
11	F	Greece	1947	11	13	17	Dysarthria	L focal	R frontal abn
12	M	Greece	1946	8	13	10	Haemolysis	Gen (absence) tonic-clonic	Minor theta abn no focal abn.
13	F	Italy	1970	12	14	15	Anaemia, tremor, menstrual irreg	Generalized	'Confirmatory'
14	F	Pakistan	1946	32	34	32	Bradykinesia, rigid	?Generalized	—
—	F	UK	1953	8	8	1	Asympt (affected sib)	Febrile conv	Sl bilat abn

DISCUSSION

There may be difficulties in determining the presence or absence of seizures in a disorder in which involuntary and paroxysmal movements are a prominent feature. Epilepsy is a clinical concept and diagnoses made by physicians were accepted. To minimize false positives, only unequivocal descriptions of seizures were considered. This may have led to an under-reporting of cases, particularly of focal motor epilepsy.

As 13 patients in a series of 200 cases of WD showed epileptic manifestations, epilepsy is not a rare feature of this disease. This series constituted a large proportion of the total number of cases of WD in the UK, and there was no evidence that the sample was biased in favour of the inclusion of cases of epilepsy. The rate of occurrence of epilepsy in our series was consistent with other series, such as those of Strickland and Leu (1975) (3 epileptic cases out of 40 = 7.5%) and Xu *et al.* (1981) (3/80 = 3.75%). In addition we identified 27 cases with seizures from the 520 cases of WD (5.2%) surveyed by Cumings (1959), and 14 from 216 cases (6.5%) included in the EEG studies (Table 3).

The series contained 2 pairs of related patients—2 first cousins (Cases 3, 4) and 2 sisters (Cases 7, 14). The cousins both had similar absence seizures. The sisters were the oldest patients in the series by several years, both in terms of developing manifest WD and epilepsy. Their WD followed a similar course. One sister had focal attacks, often secondarily generalized; the other certainly had generalized seizures, but it was unclear whether there were ever any focal features. These resemblances are consistent with the notion that the predisposition to seizures has a familial basis.

The failure of the statistical analysis to associate epilepsy with more than 2 other

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Anticonvulsants	*Response to anticonvulsants*	*Relationship and response of ep to WD therapy*	*Response of WD to WD therapy*	*Pyridoxine*	*Follow-up*	*Other*
Phenobarb, phenytoin	Good	After/good	Died aged 15	Y	Y	Pyridoxine did not stop seizures
Phenobarb	Good	Before/good	Died aged 14	N	N	
Phenytoin	Good	After/good	Improved	Y	Y	Cousin of Case 4
Phenytoin	Good	After/good	Improved	N	Y	Cousin of Case 3
—	—	Before/good	Worse, then improved	Y	Y	
Phenytoin, valproate	Good	Before/good	Poor	N	N	Later had liver transplant
Carbamazepine, clonazepam	Slight	After/fairly good at 3 yrs	Improved	Y	Y	Sister of Case 14, now in Pakistan
Primidone, carbamazepine	Partial	Before/fairly good	Fair, but died aged 48	N	Y	Marked atrophy L > R on CT scan
Phenytoin, phenobarb	Poor	After/worse	Worse, died aged 21	Y	Y	
Phenytoin	Good	After/good	Improved	Y	Y	Seizures provoked by TV
Phenobarb	Good	After/good	Improved	Y	Y	Pyridoxine no effect on EEG
Phenytoin, phenobarb	Partial	Before/partial	Sl improved	N	N	Some seizures on watching TV
Phenobarb	Partial	After/partial	Worse, then sl improved	N	N	
Phenytoin	Poor	Before/partial	Sl improved	?	—	Sister of Case 7
—	—	Before/good	Remains asympt	N	Y	

Y = yes, N = No, abn = abnormality, sl = slight

variables was disappointing. The explanation is a statistical one, and related to the fact that epilepsy occurred in less than 10% of the total sample. The majority of epilepsy cases had neurological symptoms predominantly, but of no particular pattern and not more severe than in other patients. The association with incongruous behaviour was interesting, although the nature of the relationship was unclear. As the emergence of epilepsy in WD was not clearly related to the severity of symptomatology, other factors may be involved in its pathogenesis.

Our findings confirm that epilepsy can be among the very early manifestations of WD, sometimes preceding other neurological symptoms by years, such as in Case 6. More often, however, epilepsy is associated with the development of neurological symptomatology. With regard to treatment, it may appear before or fairly soon after chelating agents are started. The prognosis of epilepsy seems to be more affected by treatment of the WD than by anticonvulsants, although both measures seem to be indicated in most cases.

One of the cases in this series, and 1 case in the literature (Bridgman and Smyth, 1944) had convulsions in early childhood, associated with febrile illnesses. An individual who has the genetic abnormality of WD and who has had febrile convulsions might be presumed to be at high risk of later epilepsy, but this cannot be demonstrated from our data, as neither of these cases had seizures later on.

The prevalence of epilepsy in this series on December 1, 1986 was 6.2% (10/161 cases surviving), while the prevalence of epilepsy in the general population is 0.62% (Hauser and Kurland, 1975). Thus in our series of WD, the prevalence of epilepsy exceeds that of epilepsy in the general population by a factor of ten; hence it is

TABLE 5 COMPARISON BETWEEN EPILEPSY ASSOCIATED WITH WILSON'S DISEASE AND EPILEPSY OCCURRING IN THE GENERAL POPULATION

	Epilepsy associated with WD	*Epilepsy in the general population*[a]
1. Rate of occurrence		
Prevalence (%)	6.2	0.62
Approx. annual incidence/1000/yr	4.3	0.54
2. Classification[b]		
Focal (%)	57	66[c]
Generalized (%)	36	34
Unknown (%)	7	0
3. Prognosis[d]		
At 6 yrs	37.5	42
At 7 yrs	60	51
At 8 yrs	60	55
At 10 yrs	75	61
At 12 yrs	100	67

[a] Data from Mayo Clinic studies (Hauser and Kurland, 1975; Annegers *et al.*, 1979). [b] The figures for classification of seizure types in WD combine the Cambridge and literature cases [c] Differences in seizure type between WD and non-WD epilepsy, $\chi^2 = 0.46$, n s. [d] Prognosis expressed as % probability of completing 5 consecutive years seizure-free.

unlikely to represent a chance relationship. If the relationship between the 2 disorders were purely one of chance, only 1 or 2 cases would be expected in our series compared with 13 reported. An approximate annual incidence rate was eight times that in the Rochester study (4.3/1000/yr compared with 0.54/1000/yr).

The statistical proportions of types of seizure associated with WD do not differ significantly from that seen in the general population. Four of the 14 Cambridge cases (28%) had focal motor seizures (as against 10% in non-WD epileptics), but this difference is too small to be of statistical significance.

The prognosis for epilepsy associated with WD appeared better than that of epilepsy in the general population (Hauser and Kurland, 1975)—for example, 75% of our series were seizure-free for at least 2 yrs at 7 yrs after diagnosis compared with less than 40% in that report—but the later analysis of the Rochester data (Annegers *et al.*, 1979) suggested a more favourable course for non-WD seizures. Table 5 shows no clear difference in outcome between the revised Rochester data and our series. It is concluded that the prognosis of epilepsy in WD appears comparable with the best quoted figures given for epilepsy in the general population.

The issue of the mechanisms producing seizures in WD has attracted controversy. Penicillamine can induce pyridoxine deficiency thereby lowering seizure threshold (Smith and Gallagher, 1970). It appears that D-penicillamine, the isomer used nowadays to treat WD, is a less potent inhibitor of pyridoxine than the L-form.

Evidence that this mechanism is of clinical relevance in penicillamine-treated WD is scanty. Smith and Gallagher (1970) reported a patient whose psychomotor status responded to intravenous pyridoxine, but not to phenobarbitone. Gibbs and Walshe

(1966) found no evidence of pyridoxine deficiency in a patient with seizures (Case 11 in this series). Walshe (1976) considered that biochemical evidence of pyridoxine deficiency is only found when another factor such as pregnancy or a growth spurt increases vitamin requirements above normal. The detection of biochemical evidence of pyridoxine deficiency (as found in a patient in our series with a single seizure) does not however establish a causal relationship.

That this mechanism is not the prime cause of seizures is further attested by those cases reported prior to the introduction of penicillamine in 1955, and by those cases in whom the onset of epilepsy precedes therapy for WD. Penicillamine is not the only agent which has been associated with early seizures: the same has been described after treatment with British Anti-Lewisite (BAL) (Hollister *et al.*, 1960) and dithiocarbamate (Passouant *et al.*, 1969).

Of the 13 Cambridge cases, 7 received pyridoxine and in these patients it did not alter seizure frequency. Case 9, whose epilepsy worsened after treatment, received pyridoxine throughout. Cases 1 and 11 were given pyridoxine on account of their seizures but control was only achieved by anticonvulsants. Our series offers little evidence that seizures in WD are related to penicillamine-induced pyridoxine deficiency.

In cases where epilepsy follows the commencement of chelating agents, the proximity in time of the two events is sometimes striking (Cases 1, 7). This cannot be due to pyridoxine deficiency but probably represents a 'paradoxical reaction'. Up to 10% of patients in the first weeks/months of therapy, show an intensification of neurological manifestations despite adequate removal of copper (Scheinberg and Sternlieb, 1984, p. 127), whereas a pyridoxine-deficiency hypothesis would predict that seizures should only arise after long-term treatment.

Copper itself can cause convulsions, certainly in animals (Davies *et al.*, 1961), probably by inhibition of membrane ATPase (Peters *et al.*, 1965; Walshe, 1981). The sudden mobilization of large quantities of copper by penicillamine may be the direct cause of the paradoxical reaction and of seizures in a proportion of patients.

CONCLUSIONS

1. In a series of 200 cases of WD, 13 had seizures at some stage. The prevalence of epilepsy in this series on December 1, 1986 was 6.2%. Both prevalence and estimated incidence rates in our series exceeded those for epilepsy in the general population by approximately a factor of ten; hence the relationship between WD and epilepsy was stronger than that expected by chance.
2. In rather fewer than half the cases seizures began before treatment of WD was commenced. Epilepsy was not often a very early or a presenting feature.
3. In about half the remaining cases, seizures began within weeks or months of starting treatment.
4. In patients receiving no therapy, seizures often occurred as a prelude to death. Median survival in untreated patients was only about 1 year.

5. The types of seizure seen in WD did not differ significantly from the findings in general population surveys, although there was perhaps an excess of focal motor seizures.

6. WD patients with epilepsy displayed more incongruous behaviour and received more medication (anticonvulsants) than WD patients without epilepsy. There were no other significant differences on a range of other demographic, clinical, biochemical and outcome variables.

7. The prognosis of WD and non-WD epilepsy seems comparable.

8. There is little evidence that penicillamine-induced pyridoxine deficiency causes these seizures. We favour the hypothesis that increased mobilization of copper by penicillamine leads directly to seizures occurring soon after the start of therapy, and we consider that cerebral copper deposition is closely linked to the epileptic manifestations of WD.

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NEUROPATHY IN MULTIPLE SYMMETRIC LIPOMATOSIS

MADELUNG'S DISEASE

by M. POLLOCK,[1] G. I. NICHOLSON,[2] H. NUKADA,[1] S. CAMERON[1]
and P. FRANKISH[2]

(From the [1]Division of Neurology, Department of Medicine, University of Otago Medical School, Dunedin, and the [2]Gastroenterology Service, Auckland Hospital, Auckland, New Zealand)

SUMMARY

With increasing age, peripheral neuropathy becomes more common in multiple symmetric lipomatosis (MSL) and the principal cause of severe disability. High alcohol consumption is frequently associated and the peripheral neuropathy of MSL is often attributed to alcoholism. In this study, sural nerve biopsies from MSL patients revealed an absence of acute axonal degeneration, a significant shift to the left of myelinated fibre diameter distributions, reduced indices of axonal and nerve fibre circularity, and an increase in myelin periodicity. This pathology supports the view that the neuropathy of MSL is not alcohol-induced but that a chronic distal axonopathy is an integral part of the MSL syndrome.

Biochemical observations suggest a defect in catecholamine-stimulated lipolysis in MSL at a membrane level, possibly in the amount or function of Gs membrane protein or in the catalytic unit of adenylate cyclase. Evidence is presented that the frequent association of MSL with alcoholism is on the basis of an additional ethanol-induced membrane lesion involving β-adrenergic receptors.

INTRODUCTION

The first clinical description of the characteristic cervical lipomata of multiple symmetric lipomatosis (MSL) was given by Sir Benjamin Brodie at St George's Hospital, London, where he described one of several cases he had seen.

> A man came to this hospital several years ago having a very grotesque appearance; there being an enormous double chin hanging nearly down to the sternum, and an immense swelling also on the back part of his neck formed by two large masses, one behind each ear, as large as an orange and connected by a smaller mass between them. They had enlarged slowly and painlessly over three or four years. (Brodie, 1846)

Madelung (1888) subsequently reviewed 30 patients from the literature and added 3 of his own. He likened the appearance of the diffuse lipoma of the neck to a ruff. It was nonencapsulated and spread diffusely, deep to the superficial fascia, along vascular sheaths and along muscular fascial planes around the pharynx and larynx. Additional symmetric lipomata usually form in the trunk and proximal limbs. The

Correspondence to Dr M. Pollock, Department of Medicine, University of Otago Medical School, Dunedin, New Zealand.

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syndrome was more frequent in males and usually associated with alcoholism. The disorder has a high prevalence in Italy and in a systematic study of 19 Italian male patients, Enzi (1984) first recognized the frequent association of severe neuropathy and MSL.

In this clinical and morphological study we have sought to determine whether the neuropathy seen in patients with MSL is an integral part of the MSL syndrome or is merely secondary to alcoholism. We have also sought an explanation for the frequent association of the MSL syndrome with alcoholism.

CASE REPORTS

Case 1

A 58-yr-old retired Caucasian seaman gave a 5½ yr history of progressive wasting and weakness. At 52 yrs of age he had noted that with exercise his calf muscles would painlessly harden and 'seize up'. Distal leg weakness rapidly ensued and 6 months later he had bilateral foot drop. At 55 yrs of age he first experienced arm spasms induced by movement and followed soon after by progressive arm weakness. Despite immunosuppressive therapy (azathioprine) and plasmapheresis he continued to deteriorate. At the age of 58 yrs he became confined to a wheelchair although still able to dress and feed himself.

He had first become aware of a lump on the right side of his neck at the age of 45 yrs. Three months later similar lumps developed on the left side of his neck and above the sternal notch. On examination 2½ months later additional lipomata were seen in the supraclavicular fossae and in the scapular regions. Subsequently lipomata formed suprapubically and along the posterior axillary folds. The cervical lipomata progressively and diffusely enlarged to produce a 'ruff-like' deformity. He gave no family history of cervical soft tissue enlargement or of neurological abnormality. He had drunk on average 0.75 l of sherry daily for 35 yrs but in the last 5 years had markedly reduced his alcohol intake. He took no drugs.

Examination revealed a massive cervical lipoma extending over his shoulders in a cape-like distribution (fig. 1). Symmetric smaller lipomata were present suprapubically and along the posterior

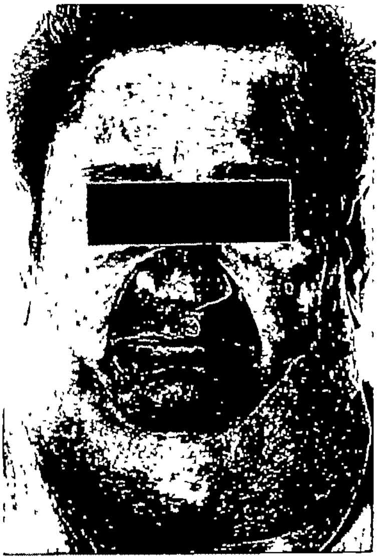

FIG. 1 The characteristic ruff-like deformity of multiple symmetric lipomatosis (Madelung's disease) in a 58-yr-old man (*Case 1*) who presented with a progressive neuropathy.

border of the arms. On examination of the cranial nerves there was mild bilateral facial weakness and moderate weakness of both sternomastoid muscles. He had marked symmetric limb wasting without fasciculations or tremor. He was able to abduct his shoulders through only 15° and could not abduct his fingers. Neither heel could be drawn up the bed and the toes moved through only 5°. The limbs were hypotonic distally with pitting oedema present in both feet. The tendon reflexes were absent, save for the left triceps and both knee reflexes. He had slight impairment of light touch, temperature and pin prick appreciation in the feet with absent vibration sense in the left great toe. Two-point discrimination was preserved in the hands and position sense was intact. He had normal sexual potency, bladder and bowel function and sweating. His casual blood pressure was 100/55 mmHg without postural drop on standing. The remainder of his neurological and general physical examination was unremarkable.

TABLE 1 SENSORY NERVE CONDUCTION

	Median nerve			*Sural nerve*	
Case	*DL*	*CV*	*Amp.*	*CV*	*Amp.*
1	3.1	56	9	NR	NR
2	4.4	ND	10	ND	ND
3	3.4	51	15	44	8
Normal	< 3 5	> 56	> 25	> 41	> 15

DL = distal latency (ms); CV = conduction velocity ($m{\cdot}s^{-1}$); Amp = amplitude (μV); NR = no response; ND = not done.

Electrodiagnostic studies showed absent sural sensory potentials but normal sensory conduction velocity in the median nerves although the action potentials were reduced in amplitude (Table 1). Needle electromyography demonstrated chronic denervation in tibialis anterior, vastus lateralis and biceps muscles. Biopsy of the left quadriceps muscle showed fibre type grouping of both atrophic and hypertrophic type I and type II muscle fibres without necrosis or inflammation. The right whole sural nerve was biopsied under local anaesthesia in November 1984.

Case 2

A 65-yr-old retired barman gave an 8 yr history of progressive wasting and weakness of his limbs. He had experienced difficulty in getting out of chairs, in climbing stairs and in picking up articles from the floor. On several occasions he had fallen and been unable to get up unaided. He had complained of numb feet and calf cramps. He first developed lipomatous deposits in his neck at the age of 47 yrs which had slowly enlarged and caused him to lean forward. Subsequently similar deposits developed proximally in the limbs and abdomen. In three surgical procedures he had lipomata removed from the nape of his neck, the left supraclavicular fossa and the posterior aspect of the left upper arm.

He suffered pulmonary tuberculosis at the age of 33 yrs requiring a right thoracotomy at the age of 35 yrs and treatment with antituberculous therapy until the age of 39 yrs. A year later he had both haematemesis and melaena from a gastric ulcer which responded to conservative treatment. Since the age of 51 yrs he had had several attacks of gout. There was no family history of similar fatty deposits or of neuropathy. He had started drinking at the age of 15 yrs and had averaged at least 5 l of beer per day until his retirement 4 yrs previously, when his intake had reduced to 1.5 l of beer per day. He had a good appetite and his weight was stable at 79 kg.

Examination revealed a plethoric, anxious, quarter-caste Maori with a massive cervical lipoma extending in a cape-like distribution over his shoulders. Similar lipomata were noted proximally in the arms, in the hypochondrium, the lumbar region and suprapubically. There was by contrast marked distal wasting and hypotonicity in the arms and legs. The peripheral nerves were neither enlarged nor tender. Severe weakness (MRC grade 3) was found in the hands but only mild weakness (MRC grade 4) proximally in the upper arms and in the legs. The tendon reflexes were present except at the ankles. He had a glove and stocking sensory impairment to pin prick, light touch and temperature, 'shading off' to normal at the elbows and at the left knee and right midcalf respectively. The remainder of the examination was normal.

Electrodiagnostic studies showed distal sensory latencies prolonged and sensory action potentials reduced in amplitude (Table 1). Motor conduction velocity was mildly reduced in the common peroneal nerves and at the lower limit of normal in median nerves (Table 2). Needle electromyography demonstrated chronic denervation in the vastus medialis, biceps and first dorsal interosseous muscles. Biopsy of the left whole sural nerve was undertaken in November 1984.

TABLE 2 MOTOR NERVE CONDUCTION

	Median nerve			*Ulnar nerve*			*Peroneal nerve*		
Case	*DL*	*CV*	*Amp.*	*DL*	*CV*	*Amp.*	*DL*	*CV*	*Amp.*
1	4.2	50	2	ND	ND	ND	ND	ND	ND
2	4.3	49	9	ND	ND	ND	6.1	29	2.5
3	ND	ND	ND	2.5	62	13	4.3	43	7
Normal	< 4.2	> 50	> 5	< 3	> 51	> 6	< 6.6	> 41	> 2

DL = distal latency (ms); CV = conduction velocity ($m \cdot s^{-1}$); Amp = amplitude (mV); NR = no response; ND = not done.

Case 3

A 55-yr-old single man with a Spanish mother and an Italian father was admitted with a 3 month history of burning painful feet. His pain had progressively worsened in intensity, was exaggerated by walking but ameliorated by alcohol. He had recently noted a numb patch on the dorsum of the left hand and intermittent numbness of the right middle finger. Friends had commented that his right upper eyelid drooped. He had no other complaints and no symptoms to suggest autonomic disturbance.

He had been admitted to hospital 2 years previously with haematemesis and melaena. Endoscopy had shown an oesophageal stricture, a hiatus hernia, gastritis and duodenitis. He had responded to ranitidine treatment. He had begun drinking alcohol at the age of 15 yrs and throughout much of his early life had drunk brandy 'like water'. Recently he had consumed 0.75–1 l of sherry per day.

On examination he had a diffuse cervical lipoma extending in a 'cape-like' distribution over his upper trunk. Lipomata were also present in the lumbar and periumbilical areas. The liver was palpable 2 cm below the costal margin in the midclavicular line, but there were no stigmata of chronic liver disease. Neurological examination revealed normal intellectual function but a complete right Horner's syndrome. He had no weakness or wasting, but both ankle jerks were absent. There was mild symmetric intention tremor on finger-nose and heel-shin testing, and his gait was 'careful' because of a sensitivity of his soles. In the upper limbs light touch was impaired in the distribution of the left superficial radial nerve and temperature sensation diminished over the fingertips. In the legs, light touch, pin prick and temperature sensation was impaired distal to the ankles, vibration sense diminished in the toes, but position sense was normal. Peripheral nerves were neither enlarged nor tender.

Electrodiagnostic studies revealed reduced sural and median sensory action potentials, mild slowing of median sensory conduction velocity (Table 1) and an absent tibial H reflex. Motor conduction velocities were normal. Needle examination showed chronic denervation in the first dorsal interosseus and tibialis anterior muscles. Biopsy of the right whole sural nerve was undertaken in September 1986.

Results of other investigations

Negative or normal laboratory tests in the 3 patients included a full blood count, erythrocyte sedimentation rate, antinuclear factor, blood urea, serum creatinine, serum electrolytes, fasting blood glucose, serum vitamin B_{12}, serum folate, alkaline phosphatase, total serum protein and protein electropheresis, thyroid function tests, serum lead, CSF examination and urinary porphyrins.

In Cases 2 and 3 serum γ-glutamyltransferase was elevated (> 900 IU/l).

HISTOLOGICAL METHODS

Seven controls (aged 36–64 yrs) for nerve biopsy were selected on the basis of a normal neurological evaluation and normal nerve conduction studies. Informed consent for sural nerve biopsy was obtained from both controls and patients.

The processing of sural nerve tissue and its morphometric evaluation were carried out according to previously described methods (Pollock *et al.*, 1983). In short, total fascicular area was obtained by digitization of photographic prints (× 300). Myelinated fibre diameter histograms were obtained from photographic prints (× 1200) using a particle size analyser (Zeiss TGZ-3) set on an exponential scale. At least 1000 myelinated nerve fibres were counted in each specimen to give a myelinated fibre density per whole nerve. Unmyelinated axon counts were undertaken from 4 or more different fascicles, from an area comprising at least 15% of the total fascicular area, at a magnification of × 14 000. Unmyelinated axons were distinguished from Schwann cell profiles by their round or oval shape, lighter appearance, higher number of microtubules and the presence of mesaxons. In each nerve, a diameter histogram of at least 300 unmyelinated axons was obtained from photographic enlargements (× 12 000), using a Zeiss TGZ-3 particle size analyser set on a linear scale. Diameter histograms of both myelinated nerve fibres and unmyelinated axons were compared with controls using the Kolmogorov-Smirnov test (Siegel, 1965).

Ultrastructural morphometry was undertaken on a minimum of 100 myelinated nerve fibres as encountered serially in X and Y traverses. Using a film strip projector, 35 mm electron microscopic negatives were back projected and axonal and nerve fibre perimeters measured with a computerized X-Y digitizer. The number of myelin lamellae were then counted on these negatives. Computer programming was used to determine myelin periodicity, indices of circularity (Dyck *et al.*, 1980), and myelin spiral length. Regression analysis was undertaken of number of myelin lamellae versus axonal area (log 2_e).

For teased nerve fibre analysis, 100 myelinated fibres with a minimum of 4 internodes were teased and graded (Pollock and Dyck, 1976). The kurtosis of the binomial distribution was used to test whether abnormal internodes were randomly dispersed amongst all nerve fibres and the χ^2 test used to assess for an increased frequency of adjacent abnormal internodes.

NERVE BIOPSY RESULTS

Patients with MSL when compared with controls showed a significant increase ($P < 0.001$) in single teased sural nerve fibres with remyelinated internodes (type F, Table 3). When all internodes were considered, MSL nerve biopsies also showed a significant increase ($P < 0.005$) in remyelinated internodes (Table 4). Statistical analysis indicated that remyelinated internodes were not randomly distributed but clustered along particular nerve fibres in MSL patients ($P < 0.001$). The incidence of adjacent remyelinated internodes was also significantly increased in MSL nerve biopsies ($P < 0.0001$) compared with controls. By contrast, changes of acute axonal

TABLE 3 GRADING[1] OF TEASED MYELINATED FIBRES IN SURAL NERVES OF PATIENTS WITH MULTIPLE SYMMETRIC LIPOMATOSIS AND CONTROLS

	Teased fibre types (%)						
	A	*C*	*D*	*E*	*F*	*G*	*Abnormal (%)*
Case 1	59	2	2	0	37	0	41
Case 2	52	0	4	1	42	1	48
Case 3	54	0	0	0	46	0	46
Controls (n = 7)	89	0	0	0	11	0	11

[1] A = normal appearance; C = segmental demyelination; D = segmental demyelination and remyelination as evidenced by at least a 50% reduction in myelin thickness; E = linear rows of myelin ovoids and balls; F = segmental remyelination; G = focal irregularity of myelin forming globules.

TABLE 4 GRADING[1] OF SURAL NERVE INTERNODES FOR EACH PATIENT WITH MULTIPLE SYMMETRIC LIPOMATOSIS AND CONTROLS

	Teased fibre types (%)				
	A	*C*	*F*	*G*	*Abnormal (%)*
Case 1	87.3	1.1	11.6	0	12.7
Case 2	84	0.4	15.2	0.4	16.0
Case 3	82.2	0	17.8	0	17.8
Controls	95.8	0	4.2	0	4.2

[1] A = normal internode; C = demyelinated internode; F = remyelinated internode; G = globular internode.

TABLE 5 SURAL NERVE FIBRE DENSITIES IN PATIENTS WITH MULTIPLE SYMMETRIC LIPOMATOSIS AND CONTROLS (per mm^2)

	Myelinated fibres	*Unmyelinated fibres*
Case 1	4894	28 768
Case 2	4734	27 888
Case 3	5693	30 434
Controls	7812	26 719
(SD)	(1630)	(3729)

degeneration (type E) and demyelination (type C) were not significantly increased in MSL nerve biopsies.

Myelinated nerve fibre densities, although at the lower limits of normal, were not significantly reduced in patients compared with controls (Table 5). A comparison of diameter distribution histograms by the Kolmogorov-Smirnov test showed a

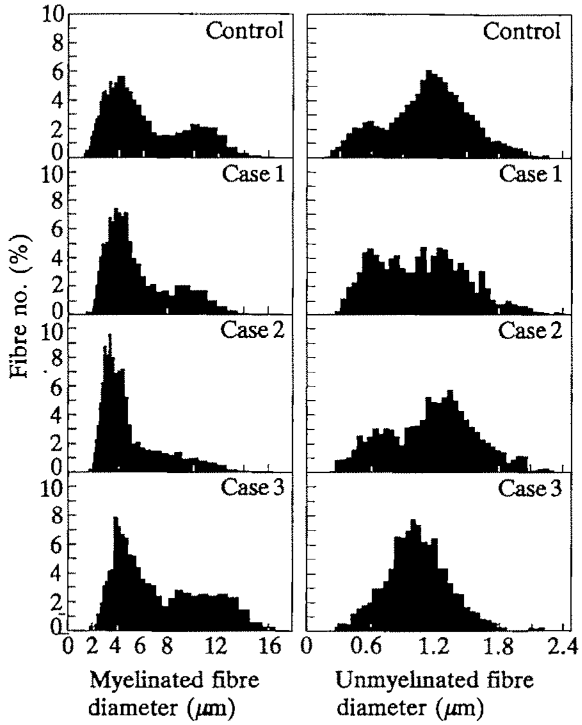

FIG 2 Diameter histograms of myelinated and unmyelinated sural nerve fibres from multiple symmetric lipomatosis patients and controls.

significant left shift ($P < 0.001$) for both small and large myelinated fibres from MSL biopsies compared with controls (fig. 2). There was no significant difference in the density of unmyelinated axons between patients and controls.

A quantitative electron microscopic analysis of myelinated sural nerve fibres showed that patients with MSL had significantly fewer myelin lamellae ($P < 0.001$), less circular axons with a decreased index of circularity ($P < 0.001$), and less compact myelin with a greater distance between myelin lamellae ($P < 0.001$) compared with controls (Table 6). The ratio of axonal area to whole nerve fibre area (axon + myelin)

TABLE 6 MORPHOMETRY OF SURAL NERVES IN PATIENTS WITH MULTIPLE SYMMETRIC LIPOMATOSIS AND CONTROLS

Subjects		*No. lamellae*	*Axonal area* (μm^2)	*Axonal index of circularity*	*Nerve fibre index of circularity*	*Myelin spiral length* (μm)	*Myelin periodicity* (*nm*)
MSL	Mean	52.0	12.3	0.76	0.84	1162	25.9
	(SD)	(34.2)	(13.6)	(0.17)	(0.12)	(1214)	(9.2)
Control	Mean	61.2	9.8	0.84	0.91	847	22.6
	(SD)	(33.6)	(9.5)	(0.19)	(0.09)	(881)	(9.3)

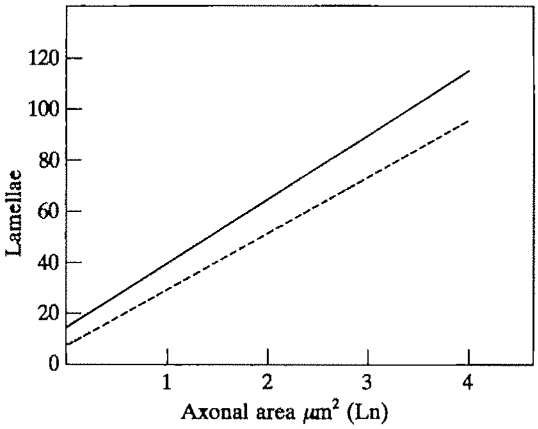

FIG. 3. Mean regression lines for number of myelin lamellae and axonal area (log to base e) in sural nerve biopsies from patients with multiple symmetric lipomatosis and neuropathy (broken line) and controls (solid line).

in the sural nerves of MSL patients was not significantly different from controls. Regression of numbers of myelin lamellae on axonal area indicated that patient and control lines were significantly separated ($P < 0.001$) although not significantly different in slope (fig. 3). Taken in conjunction with the above morphometric results it suggests that for a given axonal area MSL nerve has less myelin than control nerve. In contrast, regression of myelin spiral length on axonal area showed patient and control lines to be significantly different in slope ($P < 0.001$), suggesting that for a given axonal area MSL nerve has a longer myelin spiral length (fig. 4).

Ultrastructurally MSL nerve had frequent adaxonal honeycombing (fig. 5) unassociated with excess lipid in Schwann cells, Remak cells or fibroblasts.

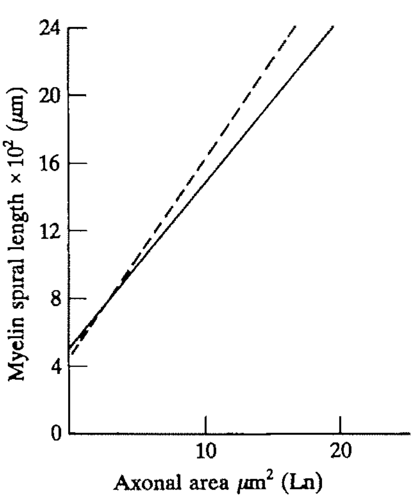

FIG. 4. Mean regression lines for myelin spiral length and axonal area in sural nerve biopsies from patients with multiple symmetric lipomatosis and neuropathy (MSL) (broken line) and controls (solid line).

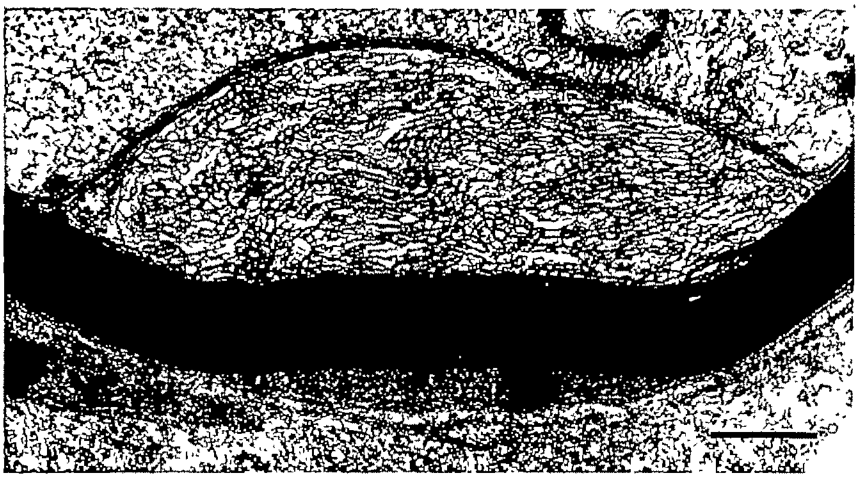

FIG. 5. Electron micrograph of a transverse section of sural nerve from *Case 2* showing a myelinated fibre with adaxonal 'honeycombing' Bar = 0.5 μm.

DISCUSSION

High alcohol consumption is often seen in MSL patients together with macrocytic anaemia and abnormal liver function tests. As a consequence the peripheral neuropathy seen in the MSL syndrome is often attributed to alcohol toxicity. There is a general consensus that axonal degeneration is the histological lynchpin of alcoholic peripheral neuropathy (Walsh and McLeod, 1970; Behse and Buchthal, 1977; Said and Landrieu, 1978). Neurophysiological findings in alcoholic subjects support the concept of axonal degeneration in peripheral nerve. Fibrillation potentials are first detected electromyographically in distal muscles and gradually become more widespread and frequent as the neuropathy progresses. There is an early reduction in amplitude of sensory action potentials in the legs (D'Amour *et al.*, 1979) but motor and sensory conduction velocities are only mildly to moderately slowed (Mawdsley and Mayer, 1965). Finally, single fibre electromyographic studies (Thiele and Stålberg, 1975) have provided evidence of reinnervation in alcoholic subjects. It is therefore important that in this nerve study of patients with MSL, no increase was found in the incidence of acute axonal degeneration despite severe clinical neuropathy. Further, in 2 of the MSL patients the neuropathy progressed despite reduced alcohol intake.

There are additional arguments against the concept of an alcohol-induced neuropathy in MSL. The incidence of neuropathy in MSL patients is unrelated to alcohol consumption with an absence of correlation between alcohol intake and motor conduction velocities (Enzi *et al.*, 1985). Moreover, neuropathy may occur in MSL patients who are nondrinkers. An alternative and more likely possibility is that the neuropathy in MSL is an integral part of the syndrome.

Our morphometric study suggests that the primary peripheral nerve pathology in

MSL is a slowly progressive axonal atrophy. Evidence on nerve biopsy, supporting this conclusion, is an absence of acute axonal degeneration or demyelination, a shift to the left of myelinated fibre diameter distributions, reduced indices of axonal and nerve fibre circularity, and an increase in myelin periodicity in MSL patients compared with controls. This concept is further supported by the finding on nerve morphometry that, compared with controls, myelin spiral length in MSL subjects is increased in relation to axonal area. Late consequences of chronic experimental axonal atrophy are clustered remyelinated internodes and ultimately a loss of nerve fibres (Dyck, 1982). This experimental evidence would thus account for the additional findings on nerve biopsy of clustered remyelinated internodes and reduced nerve fibre densities, perhaps as secondary and tertiary events in the MSL syndrome.

Against this hypothesis was the finding of significantly increased mean axonal areas in MSL patients compared with controls. It is possible that this results from a sampling artefact at the time of electron microscopy. An alternative hypothesis would be that there is, at least in part, a primary demyelinating-remyelinating pathology in the MSL syndrome.

Honeycombing of inner myelin lamellae was frequent in MSL biopsies and raised the possibility of an abnormality of peripheral nerve myelin metabolism in this disorder. This pathology was not, however, associated with increased lipid within adjacent Schwann cells, Remak cells or fibroblasts and it is possible that this myelin disturbance is also a consequence of chronic axonal atrophy.

Postmortem three-dimensional morphometric studies in abstemious MSL patients would be of value in determining whether the peripheral nerve findings in MSL result from a primary abnormality of neurons in the anterior horns and in dorsal root ganglia.

With increasing age, peripheral neuropathy becomes progressively more common in MSL and the principal cause of severe disability. Nonetheless, the course of the neuropathy is quite variable, and it may be static or slowly progressive. As typified in this report, most MSL patients with peripheral nerve involvement present with a predominantly motor or sensory neuropathy. Initial symptoms and neurological findings often suggest a multiple mononeuropathy, but by the time of admission most patients have a symmetric polyneuropathy.

Autonomic symptoms (gustatory sweating, anhidrosis, impotence) occasionally predominate, and there may be tachycardia and impaired cardiovascular reflexes (Lyon, 1910; Enzi *et al.*, 1985). This clinical and laboratory evidence of prominent autonomic involvement suggests a preferential loss of unmyelinated nerve fibres in some MSL patients in contrast to the findings in this study.

Biopsied lipomata in MSL appear histologically benign (Argenta *et al.*, 1981), although the average size of lipomatous adipocytes is reduced compared with uninvolved subcutaneous fat (Enzi, 1984). A neoplastic tendency of lipomatous adipocytes in MSL is suggested by culture studies which show a faster growth, numerous nuclear pockets and more abundant and thinner cytoplasmic microfilaments compared with normal adipocytes (Cinti *et al.*, 1983). Nonetheless, malignant degeneration of a subcutaneous lipoma has only been recognized in one individual with MSL (Tizian *et al.*, 1983). Despite the rarity of malignant transformation, mediastinal obstructive syndromes and severe neuropathy are not uncommon in MSL and the former name 'benign symmetric lipomatosis' for this disorder would now seem to be inappropriate.

A principal abnormality of lipomatous tissue in most but not all MSL patients (Pecquery *et al.*, 1980; Kather and Schröder, 1982) is a defective lipolytic response to major lipid mobilizing stimuli such as fasting and noradrenaline infusion (Enzi *et al.*, 1977). Correspondingly, lipomatous cells from MSL patients show an insensitivity to adrenergic stimulation (Enzi *et al.*, 1977; Desai *et al.*, 1979; Dorigo *et al.*, 1980; Wesslau, 1983).

Pharmacological studies have shown that adipocytes contain several types of adrenergic receptor (Motulsky and Insel, 1982). β-adrenergic receptors (primarily β_1) mediate stimulation of adenylate cyclase and increased lipolysis. By contrast, α_2-adrenergic receptors mediate an inhibition of adenylate cyclase and a reduction in lipolysis. The stimulation or inhibition of adenylate cyclase is mediated respectively by two discrete guanine nucleotide membrane proteins, Gs and GI (Spiegel *et al.*, 1985) that, like the catalytic unit producing cyclic AMP, are orientated towards the cytosol (fig. 6).

The response of MSL lipomatous tissue to the lipolytic effect of cyclic AMP is retained and under basal conditions ATP concentrations are normal (Enzi *et al.*, 1977). These observations suggest that the block in catecholamine stimulated lipolysis in MSL is at a membrane level. Alpha blockade (phentolamine) does not modify the response of lipomatous tissue to adrenaline and thus provides evidence against an anomalous prevalence of α-adrenergic receptors (Dorigo *et al.*, 1980). Lipomatous adipose tissue in MSL patients has normal numbers of β-receptors (Pecquery *et al.*, 1980; Wesslau, 1983). The primary defect in the adipocyte membrane in MSL is therefore likely to lie beyond the cell-surface receptors, possibly in the amount or function of Gs-protein, the coupler between β-adrenergic receptors and adenylate cyclase. Alternatively, the defect in MSL could be in the catalytic unit of adenylate cyclase. It is possible that similar membrane lesions in peripheral nerve account for the peripheral neuropathy in MSL.

Seminal to the discussion of pathogenetic mechanisms in MSL is the strong association of MSL with alcoholism. Recent biochemical studies have provided evidence of alcohol consumption inducing widespread membrane disorders (Harris, 1984). An important consequence of chronic experimental ethanol ingestion is a reduction in numbers of central and peripheral β-adrenergic receptors (Banerjee *et al.*, 1978). This finding probably explains earlier reports of a reduction in β-receptor response to noradrenaline during chronic ethanol treatment (Israel *et al.*, 1972; French *et al.*, 1975). It may also explain the reduction in brain noradrenaline and monoamine oxidase activity in more recent postmortem investigations in alcoholics (Borg *et al.*, 1986). Tabakoff *et al.* (1988) have provided evidence that chronic

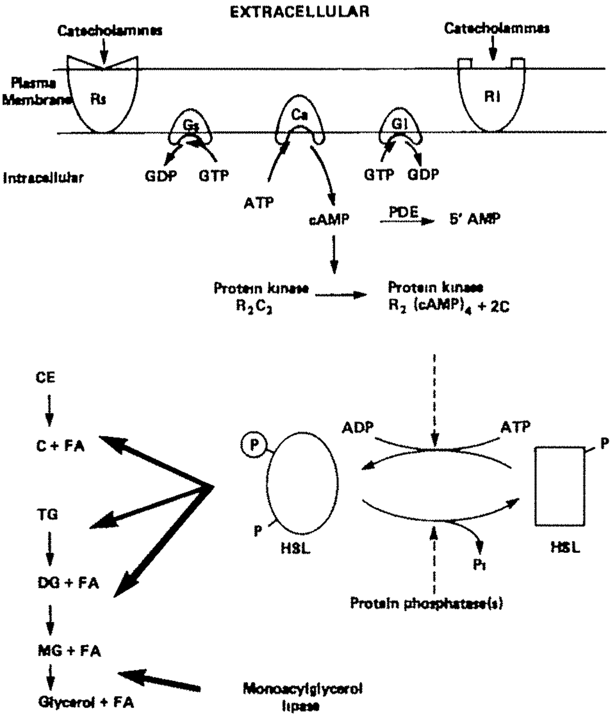

FIG. 6. Schematic outline of the catecholamine control of adipose tissue lipolysis through reversible phosphorylation of hormone-sensitive lipase (HSL). Rs and R denote stimulatory and inhibitory receptors, Gs and Gi stimulatory and inhibitory guanine nucleotide-binding proteins, GDP and GTP guanosine di- and triphosphate ATP, ADP and AMP adenosine tri-, di- and monophosphate Ca denotes the catalytic unit of adenylate cyclase, cAMP, cyclic AMP and PDE phosphodiesterase. Encircled P indicates phosphorylation of regulatory site, CE cholesterol ester, TG, DG, MG, tri-, di-, and monoacylglycerol, FA, fatty acid. (Modified from Belfrage *et al.*, 1984 and Spiegel *et al.*, 1985)

ingestion of alcohol decreases the high affinity form of the β-adrenergic receptors. The frequent coupling of MSL and alcoholism may thus be on the basis of an additional ethanol-induced membrane lesion involving β-adrenergic receptors.

The lipogenic (Lieber and Schmid, 1961), antilipolytic (Rebouças and Isselbacher, 1961) and decreased lipid oxidative effects (Jones *et al.*, 1965) of alcohol may also play a permissive role in the growth of adipocytes in MSL.

Adrenergic responsiveness is decreased during ageing in man (Krall *et al.*, 1981), and in fat cells from ageing rats (Cooper and Gregerman, 1976). The production of cyclic AMP is reduced in response to receptor agonists as well as in response to agents that act directly on the Gs membrane protein (Spiegel *et al.*, 1985). In the rat central nervous system, loss of catecholamine receptors in aged brain may be regional with a loss of β-adrenergic receptors in cerebellum and brainstem but not in the cerebral cortex (Maggi *et al.*, 1979). Studies of β-receptors on lymphocytes from elderly subjects have led to contradictory conclusions: that the number of receptors decreases with age (Schocken and Roth, 1977) or that there is no change (Davies and

Lefkowitz, 1980; Landmann *et al.*, 1981; Abrass and Scarpace, 1982). Abrass and Scarpace (1982) have provided some evidence that a decline in the activity of the catalytic unit of adenylate cyclase may be responsible for a decreased responsiveness of aged target tissues to catecholamines.

In the present context it is likely that this loss of adrenergic responsiveness with ageing is compounded by an alcohol-induced reduction in the number of β-receptors in many MSL patients, unmasking a latent lipolytic defect and accounting for the usual presentation of this syndrome in middle life.

ACKNOWLEDGEMENTS

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IDEATIONAL APRAXIA

by E. DE RENZI *and* F. LUCCHELLI

(From the Department of Neurology, Modena University, Italy)

SUMMARY

Ideational apraxia was investigated in 20 left brain-damaged patients with tests requiring the demonstration of how objects are used. On a multiple object use test the most frequent errors were those of omission, misuse and mislocation, while sequence errors were rare. Patients also failed on a single object use test, which showed a correlation of 0.85 with the multiple object use test. Neither of these tests was significantly correlated with an ideomotor apraxia test (imitation of movements). Ideational apraxia was frequently, but not exclusively, associated with damage to the left posterior temporoparietal junction.

These findings support the view that ideational apraxia is an autonomous syndrome, linked to left hemisphere damage and pertaining to the area of semantic memory disorders rather than to that of defective motor control.

INTRODUCTION

Ideational apraxia (IA) was first reported at the beginning of this century by Pick (1905) and has since been accepted as a distinct form of apraxia that is caused by the disruption of the ideational plan of action. Yet it has never acquired the same popularity enjoyed by ideomotor apraxia (IMA) and has only rarely been the subject of specific investigations. This relative neglect may in part be contingent upon its infrequent occurrence, or upon practical difficulties in testing and scoring the deficit, but mostly depends on the uncertainties that still cloud the issue of its nature and autonomy with respect to other cerebral disorders. The circumstance of having first been reported in patients suffering from dementia and confusional states and attributed to a deficit of attention (Pick, 1905) probably helped to undermine its scientific status, especially in comparison with that achieved by IMA, which Liepmann (1908) had framed in precise anatomical and physiological terms. Although it was subsequently recognized by several authors, including Liepmann (1920), that IA could also occur after focal lesion of the left hemisphere, its original doubtful status has never been completely countermanded and for several neurologists (Dejerine, 1914; Denny-Brown, 1958) IA remained just an aspect of a general disorder of brain function, secondary to deficits of attention, memory and mental associations and with little localizing value.

Correspondence to: Dr E De Renzi, Clinica Neurologica, Via del Pozzo 71, Modena 41100, Italy.

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Other authorities (Sittig, 1931; Zangwill, 1960) have challenged the autonomy of IA, maintaining that it may be just an aspect of a severe form of IMA. They argued that in many instances it would be hard to establish whether a wrong gesture is to be attributed to an ideational or an executive derangement. For this very reason, the way of testing has become critical in discriminating between the two forms: IMA is thought usually to be mildest when the patient handles an object, because of the aid provided by somaesthetic cues, while IA would be brought out best by tasks involving more than one object, since they require a detailed planning of the sequence of gestures. It is not altogether clear, however, whether actions with multiple objects are necessary, or simply more suitable to bring out IA. The former view was advocated by Poeck and Lehmkuhl (1980), who studied 4 patients with a series of multiple action tasks and argued that a major component of IA is a sequencing disorder in the conceptual organization of complex actions. This hypothesis was not borne out, however, when the performance on tests specifically tapping the ability to order actions was assessed in patients who had failed a typical IA test, such as lighting a candle (Jason, 1983). Moreover, Pick (1905) himself had also reported gross errors with single objects and De Renzi *et al.* (1968) replicated this finding when they systematically investigated the ability of left brain-damaged patients to demonstrate the use of objects. These authors were therefore inclined to revive Morlaas' (1928) interpretation of IA as 'agnosia of usage', that is, as a deficit affecting that particular aspect of object recognition that concerns the way the object must be used. In this perspective, using actions involving more than one object is merely a condition favouring the appearance of IA, and not a necessary element for its elicitation.

The many uncertainties still clouding the issue of IA are to a large extent attributable to the scarcity of detailed clinical studies and the consequent dearth of sound data on which to base the interpretation of the disorder. In the period following the Second World War, 1 case was reported by Zangwill (1960), 2 by Brown (1972) and 4 by Poeck and Lehmkuhl (1980). This may give the impression that this form of apraxia is exceedingly rare, a conclusion at variance with the outcome of the only systematic quantitative study (De Renzi *et al.*, 1968) carried out on this topic, which found IA in 28% of the left hemisphere population examined.

The present investigation was designed to fill this deficit by investigating how a group of left brain-damaged patients executed customary actions requiring the combined use of more than one object, similar to those used by Poeck and Lehmkuhl (1980). It was our purpose to collect a sufficient number of instances of faulty performances to permit an analysis and classification of errors so as to gain insight into the nature of the disorder. Therefore we did not test an unselected sample of left brain-damaged patients, but focused on those that were a priori considered likely to show IA. Their ability to execute gestures in other experimental conditions was also tested.

MATERIALS AND METHODS

Subjects

The subjects of this investigation were 20 right-handed left brain-damaged patients, 16 of whom had sustained a cerebrovascular lesion, and 4 had a tumour. Patients were admitted to the study if there was at least some hint in their history or clinical examination that they might be suffering from IA. They also had to be cooperative, able to understand verbal instructions and without clinical manifestations of dementia or confusion in daily life. Formal intelligence tests were not given, because the presence of aphasia and apraxia would have prejudiced the assessment of mental abilities. Only patients without a dense paresis of the right upper limb were investigated. Nine patients were male and 11 female. In the 16 vascular patients the examination was carried out 3 days to 3 years after the stroke. Table 1 gives the main clinical features of these patients.

TABLE 1 CLINICAL FEATURES OF LEFT BRAIN-DAMAGED PATIENTS

Case	*Age (yrs)*	*Aetiology*	*Aphasia*	*CT localization*
1	63	N	W	T
2	35	V	W	P-T
3	62	V	W	P-T
4	50	V	W	F
5	56	V	W	T
6	66	V	W	P
7	52	V	W	P
8	65	V	TM	F
9	55	V	W	P-T
10	66	V	B	F
11	55	N	A	F-T
12	53	N	TS	F
13	76	V	A	O
14	64	V	W	Neg.
15	63	V	TS	F
16	59	V	A	P-T
17	64	N	A	P
18	48	V	W	Basal ganglia
19	30	V	TM	P+SMA
20	56	V	W	P-T

V = vascular lesion, N = neoplastic lesion. W = Wernicke's aphasia; B = Broca's aphasia; A = anomic aphasia, TS = transcortical sensory aphasia; TM = transcortical motor aphasia F = frontal; O = occipital; P = parietal; T = temporal; SMA = supplementary motor area.

Tests

The following tests were administered and videotaped for subsequent analysis.

Multiple object test. The patient was requested to carry out the following five actions. (1) To light a candle. A candlestick and a candle were laid horizontally on the table together with a matchbox. (2) To open and then close a padlock. A padlock and its key were given. (3) To drink a glass of water. A Coke bottle, filled with water and covered with a cap, was presented together with a glass and a bottle-opener. (4) To prepare a letter ready for mailing. The patient was given a letter written on a sheet of paper, an envelope and a sheet of stamps. (5) To prepare an expresso pot. The expresso pot, a can of ground coffee and a pitcher of water were put in front of the patient.

The patient was prompted and encouraged if he/she appeared to be in trouble and about to give up, and the examiner eventually pointed to an object, if it was persistently neglected, but never specifically indicated how to perform the action. He insisted that the action had to be carried out as in everyday life, namely, that the letter had to be ready for mailing, the coffee pot for preparing a good cup of coffee, etc. If the patient had a moderate to severe impairment of upper limb motor function and did not succeed in executing an action that required bimanual coordination (e.g., in unscrewing the expresso pot), the examiner assisted him/her by steadying the object or completing the started action. This test was also given to 15 right brain-damaged patients, in hospital for an acute disease, which was vascular in 12 cases, and due to tumour in 3.

Object use pantomime. Twenty objects of common use were put on the table, one at a time, out of reach of the patient, who was required to pretend to hold and use them. If the performance was not flawless, a second trial was given. Scoring was as follows: 3 points: the pantomime is appropriate for the object; 2 points: the performance clearly resembles the correct one, but is somewhat imprecise or the patient uses a body part as the object; 1 point: the movement bears only a weak resemblance to the appropriate gesture, and is executed in the correct place, or it is correct, but carried out in a wrong place (e.g., when required to pantomime the use of the toothbrush, he/she makes the correct movement in front of the forehead); 0 point: the gesture is so wrong or incomplete as to be unrecognizable. A correct pantomime at the first presentation of the object received a score of 6. If a second presentation was needed, the score was the sum of the scores achieved in the two trials. Maximum score: 120. The poorest score found in 60 normal control patients is 101 (unpublished data).

Actual use of objects This test was given to 11 patients only. The 20 objects of the pantomime test were handed to the patient for him/her to demonstrate their use. The same scoring criteria adopted for the pantomime test were used. Normal subjects do not make errors on this test.

Movement imitation test (De Renzi *et al.*, 1980). This test was intended to assess IMA and consisted of 24 gestures (half symbolic and half nonsymbolic, half involving single finger movements and half hand and arm movements, half requiring holding a static position and half the execution of motor sequences) which were performed slowly by the examiner in front of the patient for him/her to reproduce them immediately afterwards. If the patient's performance was flawless a score of 3 was credited, if it was partially or totally incorrect the item was presented a second time, and, if failed again, a third time, the score being 2, 1 or 0, depending on whether the patient succeeded on the second presentation, the third presentation or never. The maximum score is 72. The test has been administered in this laboratory to more than 200 normal control patients, none of whom scored below 53. Therefore any brain-damaged patient achieving a score poorer than 53 was considered to show ideomotor apraxia.

RESULTS

Multiple object test

Only occasionally right brain-damaged patients made some minor self-corrected errors, basically consisting in pouring the water in the filter of the expresso pot instead of its bottom part, or in sticking the stamp on the left instead of on the right corner of the envelope. No right brain-damaged patient made more than three errors. Sixteen left brain-damaged patients made more than three errors (*see* Table 2). In order to understand the nature of their difficulty, the video tapes were repeatedly scrutinized and the following error categories were identified.

Perplexity. This behaviour was more common in the early stage of the performance, when the outline of the action had to be organized, but sometimes appeared later at the time a specific object had to be used. The patient looked hesitatingly at the objects, picked up one of them, turned it over, put it down, then tried with another object, giving unmistakable signs of not knowing what to do. Brief delays in initiating the action were not scored as perplexity. Most of the time, perplexity was eventually overcome and the patient made up his/her mind and carried out an action, whether correct or incorrect. In a few cases, he/she gave up after many fruitless attempts. It must be stressed that this behaviour occurred in relation to objects that Italian subjects use routinely in their daily life.

Clumsiness. The action was conceptually appropriate for the object, but carried out in an awkward and ineffectual way, because of poor control of skilled hand movements.

Omission. The patient forgot to carry out an action necessary for completing the sequence, for example, the stamp was not moistened, the envelope not sealed, the key not inserted into the padlock or the match not struck. Omissions that were spontaneously corrected a few seconds after they had been made were not scored as errors. Some omissions were impressive, since their consequences were immediately apparent and should have attracted the patient's attention, as when he/she kept on trying to pour the water from the bottle into the glass, without having previously taken off the cap.

Mislocation. The action performed with the object was appropriate, but carried out at an inappropriate place. For instance, the patient struck the match correctly, but tried to light the candlestick instead of the candle, stuck the stamp on the back of the envelope, or put the ground coffee into the bottom or the top part of the expresso pot instead of inside the filter.

Misuse. The object was used in a conceptually inappropriate way, for example, the bottle-opener was stirred inside the glass, the candle rubbed on the table, or the filter used to spoon the ground coffee from the can. The same label was also applied to the rare instances in which the object was handled at the wrong end and then used in a proper, though ineffective way. For example, the wick end of the candle was inserted into the candlestick or an attempt was made to strike the match at the wrong end.

Sequence errors. The patient employed an object without having carried out an action that must come before. For example, he/she sealed the envelope before having put the letter inside it, put the ground coffee into the filter before having poured the water into the bottom part of the expresso pot or struck the match before having inserted the candle into the candlestick. It is obvious that the distinction between omission and sequence errors is often arbitrary, because the latter always imply the former. We have followed the rule of considering as sequence errors those in which the omitted action was successively carried out spontaneously by the patient.

Table 2 shows the number of errors made by the patients for each category. The most frequent types of error were omissions (48), misuses (44) and mislocations (37). Perplexity and clumsiness errors occurred 26 and 25 times, respectively, while sequence errors were relatively rare (8). As shown in this Table, the degree of

TABLE 2 ERRORS ON THE MULTIPLE OBJECT TEST

Case	*P*	*O*	*Msl*	*Mus*	*S*	*C*	*Total errors*
1	1	2	3	4	0	0	10
2	1	3	2	3	0	3	12
3	1	1	0	0	2	5	9
4	0	0	1	0	0	1	2
5	2	2	2	0	0	1	7
6	0	3	1	1	0	1	6
7	1	2	2	0	1	2	8
8	2	6	0	1	1	0	10
9	0	6	0	1	1	2	10
10	0	2	3	1	1	0	7
11	0	1	1	1	0	0	3
12	0	0	1	0	0	0	1
13	6	5	3	6	0	0	20
14	2	2	4	4	0	1	13
15	1	2	3	1	0	0	7
16	4	2	8	14	0	4	32
17	2	3	1	2	1	2	11
18	1	2	1	2	1	2	9
19	2	2	1	3	0	1	9
20	0	2	0	0	0	0	2
Total	26	48	37	44	8	25	

P = perplexity; O = omissions; Msl = mislocations; Mus = misuse; S = sequence errors; C = clumsiness.

impairment varied widely, ranging from patients with only a few minor errors (e.g., the stamp was put to the left instead of the right upper corner of the envelope) to Case 16 who completely failed all 5 tasks.

The following examples give an idea of the kind of errors made by patients with severe IA.

Lighting a candle. The patient puts the candle vertically on the table, ignoring the candlestick, takes a match out of the matchbox and brings it close to the wick, without having struck it. After a while, he realizes that the match must be lighted, but strikes it on the candle near the wick. Finally he inserts the match on the top of the candle, beside the wick.

Drinking a glass of water. The patient attempts to open the bottle manually. When the examiner points to the bottle opener, he picks it up, but handles it on the wrong end, and puts it into the glass. Eventually he handles it at the correct end, and makes opening movements over the glass. He looks puzzled at the objects, opens the bottle manually and drinks from it direct.

Preparing the expresso pot. The patient raises the lid of the expresso pot and repeatedly tries to pour the ground coffee into its upper part, without having removed the lid of the can. Eventually she opens the can and pours the ground coffee directly from the can into the upper part of the pot without using the spoon. She unscrews the expresso pot and spills water first on the table, then into the filter. She looks perplexed for a long time at the two parts of the pot, then screws them together, after having added more water into the filter.

Relationship to ideomotor apraxia

The patients' scores on the movement imitation test for IMA are given in Table 3. Spearman's rank order correlation was computed between the scores on this test and the sum of errors made by each patient on the multiple object test for IA. A nonsignificant r value of 0.31 was found. Inspection of single patient's performance confirms the lack of association between the two phenomena. There were 4 patients (Cases 1, 6, 10, 14) who scored above 52 on the imitation test and therefore did not have ideomotor apraxia according to our criteria. All had unmistakable signs of IA. On the other hand, Case 20, who made only 2 minor errors of omission on the multiple object test, was severely defective (score of 34) on the movement imitation (IMA) test.

TABLE 3. PATIENTS' SCORES ON THE PANTOMIME TEST, USE OF SINGLE OBJECT TEST AND IDEOMOTOR APRAXIA TEST (IMA)

Case	*Pantomime*	*Use of single objects*	*IMA*
1	79	—	57
2	—	—	0
3	65	—	28
4	53	—	26
5	41	70	49
6	100	—	49
7	68	—	42
8	—	95	51
9	34	67	28
10	37	90	56
11	67	102	40
12	71	108	52
13	—	50	16
14	—	53	53
15	49	—	31
16	48	—	15
17	74	—	38
18	38	90	35
19	11	69	37
20	38	100	34

Case 2 was not given the pantomime test; Cases 8, 13 and 14 were unable to perform it

Pantomime test

All patients except 1 (Case 2) were given the pantomime test. It had, however, to be discontinued in 3 (Cases 8, 13, 14) because they could not be induced to pantomime the movements in any way whatsoever and kept on making comments on the objects and their use. Table 3 gives the patients' scores and shows that all patients performed below the cut-off score.

The pantomime test correlated with an r_s of -0.08 with the multiple object (IA) test and 0.44 with the movement imitation (IMA) test ($P < 0.05$).

Use of single objects

Only 11 patients were given this test. All of them made errors (*see* Table 3), although their performance was better than on the pantomime test. Generally speaking, the test was easy to score in quantitative terms, but more difficult to analyse qualitatively. This was due in part to the fleeting character of the performance and in part to the fact that the absence of a target for the action favoured the appearance of nondescript amorphous movements. In some cases the ideomotor nature of the impairment was apparent, since errors consisted of an awkward execution of actions or poor orientation in space, with preservation of the general configuration of the gesture. For example, Case 20, a severe ideomotor apraxic with only 2 errors of omission in the complex action test, showed considerable maladroitness in demonstrating the use of the fan, saw and key. Sometimes it was hard to decide whether the deficit was conceptual or executive, as, for instance, when the iron was moved up and down in the air. There were cases, however, in which it was apparent that the patients had no idea of how to use the object. Either they turned the tool over in their hands, eventually sketching an indefinite movement as if they did not know what to do with it, or made errors of misuse (e.g., combing themselves with the eraser, hammering with the key, sawing with the knife) or of mishandling (e.g., trying to write with the pen held at the wrong end). There was no indication that agnosia played any role in the patients's failure, as they often named the object that they could not use or said what it was for. This test was highly correlated with the multiple object test ($r = 0.85$, $P < 0.01$), whereas its correlation with the movement imitation test was only 0.31 (n.s.).

Locus of lesion

CT scan findings were available for all patients, but were repeatedly negative in Case 14, who had sustained a left-sided infarct during a left carotid endarterectomy. He had clinical signs pointing to a left parietal lesion, namely, acalculia, agraphia, mild anomia and lack of motor spontaneity with the right hand. He had a severe IA (13 errors). The locus of lesion of the other patients is shown in Table 1.

Frontal lesions were present in 6 patients. Three (Cases 8, 10, 15) were definitely apraxic (10, 7 and 7 errors, respectively) and had damage localized in the anterior insula, the precentral gyrus and the foot of the inferior and middle frontal gyri. Minimal to mild impairment was present with other localizations: a metastasis in the parasagittal area (Case 12: 1 error), an infarct extending from F3 to the lateral corner of the frontal horn (Case 4: 2 errors) and a slowly developing glioma of the inferior frontal and temporal white matter (Case 11: 3 errors).

Temporal lesions were found in 2 patients. An infarct and a glioma extensively involving the lateral surface of the temporal lobe were associated with 7 (Case 5) and 10 errors (Case 1), respectively.

Parietal lesions were found in 8 patients. In 3 (Cases 6, 7, 17) the inferior part of the posterior parietal lobe was encroached upon by the lesion. All were apraxic (6, 8 and 11 errors). In a fourth patient (Case 19) there was a second infarct in the supplementary motor area. She made 9 errors. The inferior parietal lobe and, in addition, the adjacent part of the temporal lobe was involved in 5 patients. IA was severe in 4 (Cases 2, 3, 9, 16: 12, 9, 10 and 32 errors, respectively) and practically absent in 1 (Case 20: 2 errors).

An *occipital lesion* was present in 1 patient (Case 13) who had a left posterior cerebral artery infarction. He had severe IA (20 errors). Despite the fact that he showed some difficulty on an object recognition test (and even more so on a photograph recognition test), the attribution of his impairment for carrying out complex actions to visual agnosia was unconvincing. The objects were handled, thus providing somaesthetic information, and were frequently correctly named. Furthermore, the nature of the errors pointed to an impairment occurring at a stage beyond that of mere recognition. His behaviour on the candle item is illustrative of this contention. He first tried to light the match holding it by the wrong end and, when he finally struck it correctly, he blew it out instead of lighting the candle. In a second attempt the match was held between the fingers until he burnt himself; a third match was struck and laid down on the top of the candle close to the wick. None of these errors can be accounted for by assuming a faulty recognition of the match.

Lesions of the basal ganglia were found in 1 patient (Case 8) who had a lenticular haematoma. She made 9 errors.

DISCUSSION

The nature of the deficit

The analysis of the patients' behaviour on the multiple object test provides clear support for the thesis (De Renzi *et al.*, 1968; Poeck and Lehmkuhl, 1980) that IA is an autonomous disorder and not an extreme form of IMA, as suspected by Sittig (1931) and Zangwill (1960). It is true that, if sufficiently severe, IMA can produce an awkward and ineffective use of tools and even result in amorphous movements, but these errors represent a minority in comparison to those of omission, misuse or mislocation, which reflect a defective idea of how the object must be used. The patient who puts the stamp inside the envelope, the ground coffee into the bottom part of the pot, or strikes the candle instead of the match does not fail because he/she chooses a defective innervation pattern to implement the movement, but because the sight and handling of the object do not evoke the specific action which should be associated with it. The lack of a significant correlation between the number of errors on the IA (multiple object) test and the score on the IMA (movement imitation) test bears out the conclusion drawn from qualitative analysis.

Morlaas (1928), who strongly advocated the autonomy of IA from IMA and indeed questioned its inclusion in apraxia ('l'apraxie ideatoire n'est pas, au fond, de l'apraxie', ideational apraxia is not, after all, apraxia) proposed to call it 'agnosia of usage', thereby stressing the deficit of a specific aspect of object recognition, that concerning how it must be used. We believe that the term agnosia may cause misunderstanding and should be better replaced by amnesia of usage, which clearly conveys the idea that the patient's failure is contingent upon the defective activation of the memories of actions. A similar interpretation had already been advanced by Marcuse (1904), who spoke of 'amnesic apraxia'.

Considered in this perspective, IA would pertain to the area of conceptual rather than of motor disorders. Poeck (1985) spoke of a deficit in the conceptual organization of actions 'requiring the use of various objects in the correct way and order necessary to achieve an intended goal' and stressed that 'the most frequent type of error is sequential in nature'. Actually, sequence errors were rather infrequent in our patients, and were outnumbered by those of misuse and mislocation, which are due to the incorrect use of the single object. Moreover, errors of misuse did not occur only during the performance of complex actions, but also when the patient was tested with single objects. It is worth remembering that Pick (1905) himself had pointed out this behaviour in his first case of IA, who used a razor as a comb and scissors to write.

The conclusion to be drawn from these findings is that IA is not necessarily associated with complex actions and can cause a breakdown in the execution of a gesture at any time an action must be evoked and not simply imitated. In principle, even the inability to carry out pantomimes or symbolic gestures, such as to salute or to make the sign of the cross—traditional manoeuvres for testing IMA (Goodglass and Kaplan, 1963; Geschwind, 1975)—may sometimes be due to an evocation defect and attributed to IA. This may be so when the patient remains perplexed and does not do anything, although manifesting his understanding of the instructions. It remains true, however, that for practical reasons the most effective and reliable way to assess IA is to request the patient to use multiple objects. On the one hand, errors are more frequent with complex than with simple actions, possibly because of the heavier load placed on memory and attentional resources by the former than the latter task. On the other hand, it is easier to discriminate between conceptual and executive errors when the goal to be attained requires the coordinated use of more objects than when a single action, devoid of pragmatic effects, must be performed. As Sittig (1931) repeatedly stressed, it may be difficult to classify the incomplete or amorphous movements made by the patient while handling a tool as ideational or ideomotor errors. This is even more true with pantomime, or symbolic gestures, whose interpretation is further confounded by the uncertainty about the patient's comprehension of the verbal command.

Although we concur with Foix (1916), Morlaas (1928) and Poeck (1983) in drawing a sharp line of demarcation between IA and IMA and construing the former as pertaining to the area of cognitive rather than motor disorders, it is not our purpose to dissolve its autonomy into the broad concept of dementia or confusion.

The finding that IA occurs in patients with focal lesions of the left hemisphere who do not show clinical signs of disorientation for time and place or of mental deterioration argues against such a reductionistic view. The claim (Pick, 1905) that patients fail because they are unable to focus on the task is contradicted by their behaviour that often displays a serious effort to overcome difficulties, through trial and error, and may ultimately lead to attaining the goal. The cognitive deficit is specific and concerns the ability to gain access to the semantic repository where the multiple features defining an object are stored, among which there is the way it must be used. There are other examples of the difficulty exhibited by a few left brain-damaged patients to retrieve from semantic memory an essential element of a stimulus in response to its presentation. Such patients may fail to associate an object with its colour (De Renzi and Spinnler, 1967), or its sound (Spinnler and Vignolo, 1966), or the pantomime showing its use (Gainotti and Lemmo, 1976), are at a loss when requested to draw it from memory (Gainotti *et al.*, 1983) and, more generally, meet with difficulty in appreciating the conceptual relationships linking it with other objects (Gainotti *et al.*, 1979). The bearing of aphasia on these disorders has been the subject of many speculations (Cohen *et al.*, 1983; Gainotti *et al.*, 1986), and has not yet been settled. However, an interpretation in terms of the unavailability of verbal labels would be an oversimplification, since none of the tests used in these studies involves verbal responses. Viewed in this perspective, the assumption that the basic deficit of IA resides in the lack of access to a specific aspect of the semantic store gains plausibility. It remains to be seen what degree of correlation links the different manifestations of semantic memory impairment.

The locus of the lesion associated with IA has generally been identified in the posterior region of the left hemisphere. Liepmann (1920) located it in the caudal part of the parietal lobe, Foix (1916) in the territory of the temporal angular artery, while Morlaas (1928) emphasized the damage to the superior temporal gyrus. Hécaen (1972), though agreeing on the crucial role of the temporoparietal junction, stressed the importance of the size of lesion. Our data are not suited to draw definite inferences on the relative contribution of discrete cerebral areas to IA, since they have been collected in a highly selected sample. They point out, however, that the temporoparietal junction represents the most frequent but not the unique anatomical correlate of IA. In 3 cases the disease encroached upon the posterior part of F1 and F2, in 1 case it involved the lentiform nucleus and in 1 the medial occipitotemporal junction. The issue deserves further investigation.

A final question concerns the impact of IA on everyday life. Poeck (1985) contrasted IMA, a symptom usually apparent in the testing situation alone, with IA, which would also appear in spontaneous behaviour. Our data suggest that this is true in the most severe cases, in which clinical observation and information from relatives pointed to the impairment of activities such as eating, washing oneself, shaving, cooking, etc. In milder cases, however, the disorder may not be apparent in daily life and needs the artificial conditions of testing for its demonstration.

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DISTANT REFERRAL OF CUTANEOUS SENSATION (MITEMPFINDUNG)

OBSERVATIONS ON ITS NORMAL AND PATHOLOGICAL OCCURRENCE

by G. D. SCHOTT

(*From The National Hospital for Nervous Diseases, Queen Square, London*)

SUMMARY

Certain normal individuals when scratching a small area of skin often experience simultaneously an additional punctate sensation (Mitempfindung) at a remote site on the body. Four patients are described with acquired Mitempfindungen, the sensation being referred to the region that had been rendered abnormal by damage to the nervous system. The differences between normal and pathological Mitempfindungen and other patterns of cutaneous referral are considered, and mechanisms underlying Mitempfindungen, including the possible role of the spinocervical tract, are discussed.

INTRODUCTION

> And it has been frequently observed, that when some part of the Body has upon itching been gently scratched by the Nails, there has at the same time been felt in a distant part, a very pungent *Stimulus* or Sensation answering exactly Stroke for Stroke to the Action of the scratching Nails. Thus particularly on scratching a small Pimple, a little below the right Knee on the outside, a like pungent Sensation has been felt on the left Shoulder Blade, and sometimes on that Arm some Inches below the Shoulder: And *vice versa* the right Shoulder or Arm has in like manner been affected, when the scratching has been made near the left Knee, but this Effect does not always follow: There are many Instances of the Sympathy of the Nerves.
>
> Hales (1733)

In the same volume as the earliest account of blood pressure measurement, Stephen Hales in 1733 gave the above description of the phenomenon of reference of a cutaneous stimulus which was also felt at the same time at a remote site. Features characteristic of the condition are the simultaneous experience of the initiating and referred sensations, and the apparently bizarre and inexplicable topographical relationship between the two sensations. About a century ago more extensive observations began to be made by Kowalewsky (1884), who observed and recorded the phenomenon on himself. The term Mitempfindungen, which had first been introduced by Johannes Müller (1844), began to be used in this context. While

Correspondence to: Dr G D. Schott, The National Hospital for Nervous Diseases, Queen Square, London WC1N 3BG, UK.

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nosologically unhelpful, the term Mitempfindung(en) (meaning, associated sensation(s)) is useful as a convenient term for this clinical phenomenon and has the benefit of a long period of usage; indeed, the term was used by Sherrington in 1900.

Although some variability in the clinical features emerges, Mitempfindungen are generally considered to be sensations experienced by healthy individuals. In the present contribution, it is proposed to review the clinical features of Mitempfindungen, to describe for the first time cases in which Mitempfindungen appeared as an acquired phenomenon in patients with comparatively mild painful neurological damage, and to compare both these forms of Mitempfindungen with other forms of cutaneous reference.

A few authors have made more or less detailed studies of the phenomenon and these are considered below (Kowalewsky, 1884; Mittelmann, 1920; Sterling, 1973; Evans, 1976; Richter, 1977; Kanazawa, 1978; Bean, 1979). Others have made brief observations. Weir Mitchell (1895), for instance, recounted that a friend of his had a mole on one leg which when rubbed or pinched was at once felt as a sharp pain in the chin; de Fromentel (1888) reported that scratching or evacuation of a pustule to the left of the sternum was felt as a momentary pain in the left lumbar region; Monro (1898) reported a man in whom pressure on a certain part of the left leg frequently induced pain or soreness on the left side of the chest; and Livingston (1943) could invariably elicit a tingling sensation in the left medial epicondyle region by scratching a small mole at the anterior costal margin, and recalled a friend who produced a sharp stinging pain on the anterior chest on stimulating a particular spot on the knee.

It is clear from those authors who have studied the phenomenon more extensively that Mitempfindungen are commoner than might be supposed. Thus Mittelmann (1920) reported 8 of 9 people questioned had experienced Mitempfindungen. Livingston (1943) noted 'many individuals have discovered that stimulation of certain parts which have no obvious relationship can induce pain stimuli elsewhere'. Sterling (1973) found the condition to be immediately recalled by about half of 20 individuals questioned, Evans (1976) reported that 8 of 41 unselected individuals experienced them, and Bean (1979) noted the phenomenon had been 'observed in many people'. Sinclair (1949), in a study of experimentally-induced referred cutaneous pain, found 8 of 30 normal individuals in whom pain was referred elsewhere on the skin. The frequency with which Mitempfindungen occur in a susceptible individual is obviously difficult to ascertain, depends on self-observation, and appears to be variable. For instance, Evans (1976) noted the phenomenon on 158 occasions over 35 years, Richter (1977) on more than 100 occasions over 5 years, and Bean (1979) made 'many dozens of observations' over 38 years. Those who experience Mitempfindungen seem to do so for much if not all of their lives, and the topographical features may be very stable (*see* below). Genetic studies have not been forthcoming, although Bean (1979) mentions that his 3 children as well as himself experienced Mitempfindungen whereas his wife did not.

While the condition appears to be somewhat heterogeneous, certain features are characteristic and make the phenomenon easily recognizable. The initiating stimulus is usually that of a scratch or some similar localized firm and generally innocuous stimulus applied to a very small area of skin, or irritation of or pulling on a hair follicle. It is clear that a certain intensity of skin irritation is required to elicit the phenomenon, implying that a definite threshold must be reached. Many authors report minor skin excrescences, pustules and infections, as well as warmth and moisture, are predisposing factors for inducing Mitempfindungen; inattention while scratching the skin also makes the phenomenon more likely to appear (e.g., Kowalewsky, 1884; Mittelmann, 1920; Bean, 1979). If firm pressure on a hair produces reference of sensation, removing that hair abolishes the phenomenon until a new fair has formed (Sterling, 1973). Mittelmann (1920) reported various other stimuli, including localized pain and cold, could induce referred sensations. He made the interesting observations that prolonged pressure could result in hyperpathia and spread of that referred sensation, that the smaller the stimulus, the smaller the area of referred sensation, and that stimulation by localized warmth or pain had to involve less than 1.5 mm diameter of skin. Sinclair (1949) also found that trigger points were usually very small (1-2 mm) but occasionally stimulation of a very narrow band of skin could result in referred sensations.

The referred sensations, the Mitempfindungen, always occur simultaneously, are short-lived and usually momentary, although Evans (1976) reported a sensation lasting up to 15 s. Mitempfindungen are often felt as an irritation, itch or pins and needles sensation and are nearly always more painful or disagreeable than the initiating stimulus. The quality of the initiating and referred sensations are therefore not identical. Mittelmann (1920) reported that a degree of hyperpathia could develop in the referred area and Sinclair (1949) reported in his experimental study altered cutaneous sensibility with prolonged stimulation. Mittelmann (1920) anaesthetized the point of the Mitempfindung with local anaesthetic, and found that the phenomenon still occurred but the referred sensation was no longer sharp; this observation appears not to have been repeated. The area of referred sensation is usually small, a few millimetres in diameter, but rarely the area may extend for a few centimetres or exceptionally involve a patch up to 10 cm long (Evans, 1976).

The topographical relationship between the points of stimulus and reference is perhaps the most puzzling feature. The referred sensation is usually ipsilateral and most authors specifically draw attention to this characteristic, but rarely reference to the contralateral side has been reported (Hales, 1733; Mittelmann, 1920). Various authors have found, in relation to the point of stimulus, that the Mitempfindung may be referred caudally or cranially, in either direction, near or far and to the same or different anterior or posterior surface of the body; rarely there is an impression of a mirror distribution between the two sides, but more usually distribution is asymmetric. Other curious referral patterns have been reported, such as stimuli applied to the left thumb being referred to the undersurface of the tongue (Evans, 1976). Kanazawa (1978) described referral from the inguinal region to axilla, patella to elbow posteriorly, popliteal fossa to antecubital fossa, and toe to thumb. The same area of the primary stimulus does not cause referred sensations in the same place in different individuals, and two very close primary sites may give references far apart. The only area which is often stated neither to give rise to Mitempfindungen nor to be the site of the referred sensation is the face. There are, however, exceptions. For instance, Sinclair (1981) reports touching a specific point on the bridge of his nose produced a tickle on the upper lip, and Kanazawa (1978) reported that stimulation of an area on his cheek innervated by the second division of the trigeminal nerve produced Mitempfindungen in the ipsilateral C5–6 distribution. Rarely, 2 widely separated referred sensations may be elicited simultaneously by stimulation of a single primary site (Mittelmann, 1920; Evans, 1976; Bean, 1979). Again, the referred sensations appear to have no topographical relationship with each other or to the primary site. For example, Bean (1979) found stimulating a point above the right shoulder induced double reference to the right lateral upper arm and to the lower right paraspinal area.

Although Mitempfindungen can fatigue with short-term repetitive stimulation, the reference point tends to be consistent with repeated stimuli, and the topography of the initiating and referred points may last months (Kowalewsky, 1884), years (Sterling, 1973) or even several decades (Bean, 1979), suggesting a phenomenon that is very stable, perhaps permanent and possibly congenital.

A number of features are not encountered. Thus reversed direction of referral does not occur, so that stimulation of a referral point never generates sensation in the trigger area; there is no obvious sensory abnormality, colour change or other visible cutaneous finding at the referral site; and the patterns of referral, while unpredictable, appear never to involve the same, adjacent or contralateral segments, nor to spread distally down a limb (as so typically seen in referred sensations from a damaged root or peripheral nerve). There do not appear to be any significant right-left differences.

Why only certain people ever experience Mitempfindungen is unknown, but related to this curious phenomenon are the cases described here, which have an acquired basis. In the present cases, the sensation is referred to the region that has become abnormal as a result of damage to the nervous system. None of these patients had experienced Mitempfindungen before their illness and in 3 of them it was a transient phenomenon that did not recur.

CASE REPORTS

Case 1

This 74-yr-old man developed herpes zoster affecting the right T2–4 dermatomes, followed by severe postherpetic neuralgia and patchy hypalgesia with cutaneous hypersensitivity. A month after the rash had cleared, he found that for several weeks, whenever shaving (which he did with an electric razor) a very unpleasant stab of pain would be experienced in a single small area on his anterior chest within the region affected by the neuralgia, just lateral to the midline at T3. The only region triggered by shaving was 1 to 2 cm in diameter below the centre of his chin, and the only other trigger factors he found that induced pain were absent-mindedly scratching, rubbing or sometimes washing the same area beneath the chin. Repetitive contact induced pain in the chest on just the first occasion and subsequent stimuli were innocuous unless repeated after an interval.

Examination confirmed that scratching, lightly rubbing or applying vibration with a 128 Hz tuning fork to a site beneath the chin caused stabs of pain, which were localized to a tiny, particularly painful, scarred region within the T3 dermatome, as he had described. Light touch with cotton wool, tapping, pin prick or deep pressure were innocuous. A single subcutaneous injection of 2 ml 0.5% bupivacaine below the chin has permanently abolished the referred sensations.

Case 2

This 43-yr-old man developed severe neuralgic amyotrophy with patchy involvement of the right C5/6 roots Particularly affected was the distribution of the circumflex nerve, with severe weakness of the deltoid and an area of sensory loss over the lateral upper arm. A month after the onset, he frequently found that when he combed or brushed his hair on the right side of his head or scratched a particular area in the midparietal region, he would experience a momentary painful tingling localized to a very small region within the area of sensory impairment in the upper arm. It appeared to be the movement of the hair that triggered this tingling, since tapping, pricking, rubbing and vibration never caused this phenomenon. As his neuralgic amyotrophy began to improve over 2 to 3 months, the referred pain which occurred inconstantly subsided completely.

Case 3

Three years ago, this 60-yr-old farmer developed herpes zoster in the second division of the left fifth cranial nerve which was followed by severe postherpetic neuralgia. The pain was unresponsive to analgesic and anticonvulsant drugs or transcutaneous electric nerve stimulation; local anaesthetic and alcohol injections and radio-frequency lesioning of the Gasserian ganglion and a stellate ganglion block proved only temporarily effective. Examination before and after these procedures showed patchy decreased cutaneous sensation over the left cheek.

Ever since the onset of his postherpetic neuralgia, he observed, initially by chance, that occasionally scratching a tiny area to the right of the vertex in an area remote from the zoster infection was felt as a momentary stabbing exacerbation of his persistent facial pain in a small area over the middle of the cheek. This was inconstant and unpredictable, but was only elicited from one tiny trigger area, and firm pressure, pin prick and vibration failed to elicit the same referred sensation.

Case 4

Three years ago this 59-yr-old man developed painful tingling of the medial 2 fingers and border of the left hand and medial aspect of the forearm. After 2 yrs he developed a similar but more widespread sensory disturbance with a burning component affecting the base of the left thumb and lateral aspect of the forearm. Examination revealed hyperpathia over the ulnar 2 fingers, thumb and medial and lateral aspects of the left forearm. There was no weakness or reflex abnormality.

Investigations, including MRI scanning of the spinal cord, were normal except for neurophysiological studies which demonstrated absence of the left ulnar nerve sensory action potential and a reduced radial sensory nerve action potential; there was no evidence of denervation or of slowing of motor nerve conduction velocity. It was considered but not proven that he had some form of postganglionic sensory plexopathy. The symptoms and signs had remained unchanged over 2 yrs.

For a period of just some weeks, he found by chance that scratching a tiny papule, possibly a hair follicle, on the left lateral side of his trunk at about the level of T8 dermatome induced a striking but momentary exacerbation of pins and needles sensations in a small area along the medial border of the forearm, each scratch inducing a shooting pain in that area. Light touch, rubbing and pin prick over the trunk did not induce these symptoms.

DISCUSSION

These 4 patients reported, unprompted, the curious phenomenon of transient referral of cutaneous sensation evoked by innocuous stimuli to a damaged area of the body in which pain or sensory disturbance was already experienced. Do these patients demonstrate a phenomenon of acquired and pathological Mitempfindungen?

The features in common are the firm but painless mechanical form of the trigger stimulus, the simultaneous timing of triggered and referred sensations, the curious and unpredictable localization of the trigger point and the inconstant but sometimes repeatable triggering. The referred sensations were, as in typical Mitempfindungen, different and somewhat unpleasant, but may have been rather more painful, and the area involved perhaps a little more extensive, than the sensations typically described. Contrasting with the unpredictable site of Mitempfindungen in normal individuals, however, sensations were referred to the already damaged and painful part, and the phenomenon was transient in 3 of the 4 patients. It therefore seems reasonable to propose that these patients demonstrate an acquired form of Mitempfindungen. How do Mitempfindungen, whether in normal individuals or as an acquired condition, differ from other examples of referred cutaneous sensation?

Between normal and pathological states of bizarre reference of cutaneous sensations is the experimental report of Sinclair (1949) referred to above. In 8 of 30 healthy volunteers, he elicited pain from various specific areas of the body, which, when subjected to stimulation by squeezing, pricking or crushing, produced referred pain in other and sometimes far distant areas. After a while an alteration in cutaneous sensibility in the referred area could sometimes occur. This phenomenon differs from Mitempfindungen in the production of the phenomenon by definitely noxious rather than innocuous stimuli, the experimental rather than spontaneous nature of the referred sensations and the change in cutaneous sensibility in the referred area. The observations do, however, demonstrate the phenomenon of the apparently bizarre topography of reference in healthy individuals. So too does the observation concerning prodromal itching in certain asthmatic children (David *et al.*, 1984). Children subject to asthma may experience itching up to 30 min before an attack and, in a prospective study, 26 of 79 children were found to have subjective itching or objective evidence of scratching. This prodromal itching, however, could occur in unusual areas including some far from the chest, such as the vertex, the umbilical area, side of the neck and umbilicus, right side of the cheek and both legs. The location of the itching was constant in each patient and in some patients more than one site was involved. Here again, a transient sensory disturbance triggered not by cutaneous stimulation but by an impending asthmatic attack, occurs in a fixed and apparently inexplicable distribution in neurologically intact individuals.

Other examples exist of misreference, particularly of pain or other disagreeable sensations, and such misreference may either be persistent or transient. Apart from referred visceral pain, which will not be considered here, damage to cutaneous and deeper structures can be felt in inappropriate areas. Thus Weir Mitchell (1895) described a patient in whom a heavy weight fell on the right foot mainly injuring the toes, but immediately at the time of injury, acute pain was also felt on the uninjured left leg, and sensitivity of the left tibia and overlying local warmth were detectable. The referred pain was felt more severely and for a longer duration than the initial injury, and it is noteworthy that the site of referred pain was not in the homologous area, indicating that local segmental involvement was unlikely. He also describes other patients, including a man with an injury to the right thumb, but with pain experienced simultaneously in the left thumb; a patient with an injury to a finger which was associated with brief and intense pain on the contralateral side of the face and neck; a shell wound of the right leg being experienced in both feet, right arm and chest; and a shell wound of the left thigh producing bilateral thigh pain. More recent instances have been described, including severe left arm pain in a patient with an occult chronically infected cavity in the left maxilla, the pain only subsiding with resolution of the infection (Ratner *et al.*, 1978).

More akin to Mitempfindungen because of their brevity are examples of unusual momentary misreference of cutaneous sensation, and whether indeed such examples represent true Mitempfindungen is debatable. Thus Weir Mitchell (1895) cites the report of an individual who felt an injection (site unstated) to be felt as an instantaneous pain in the head. A personal case illustrates a similar phenomenon.

> Two years ago this 40-yr-old man began to experience intermittent pain in the lower right third molar tooth. He received extensive treatment including root filling and eventually an abscess developed, which settled with antibiotic treatment. He found that his dental pain tended on occasions and unpredictably to be provoked by changes of temperature within the mouth, and when this pain occurred, he experienced a simultaneous shoot of pain in a small area in the lower thoracic region near the midline. Firm pressure, percussion and light touch of the affected and other teeth did not induce the thoracic pain, neither did chewing nor clenching the jaws. Following treatment of the dental abscess, both his dental and the referred thoracic pains disappeared.

Here the features in common with Mitempfindungen are the simultaneous timing of triggered and referred sensations, the curious and unpredictable topographical relation between the 2 sensations and the inconstant but sometimes repeatable triggering. In contrast, however, is the fact that the initiating stimulus was not the typical mechanical one but changes of temperature.

Curious patterns of cutaneous referral may also be induced in patients with major damage to the nervous system. Pain may be referred from a damaged area to a normal area: thus Nathan (1956) described patterns of referral of sensation in patients who had had a cordotomy, and in whom various stimuli applied to the area rendered more or less analgesic below the level of the cordotomy could be referred to one or more sites with normal innervation. The reference of sensation, however, differed in many ways from Mitempfindungen: referred sensations were induced by painful and not innocuous stimuli, were often reproducible, often felt on the contralateral rather than the ipsilateral side, were sometimes felt increasingly far away from the stimulus point with increasing intensity of stimulation and, in particular, the referred sensation was the only sensation felt, compared with the two stimuli felt with Mitempfindungen. Similar phenomena have been reported by various authors over the past century (e.g., J. K. Mitchell, 1895; Nagaro *et al.*, 1987).

The opposite may also occur, when sensation is referred from a normal area to within an area of abnormal sensibility. For instance, Bors (1979) described 6 of 50 patients with spinal cord injury in whom sensory stimulation at or above the level of the lesion produced localized reference of sensations as well as in the damaged segment. The referred sensations could be ipsilateral or bilateral, and were induced by innocuous mechanical stimuli. Both the initiating and referred sensations were perceived, a phenomenon Bors called synaesthesia (*see* below). More recently Katz and Melzack (1987) reported that 39% of patients with chronic pain syndromes of various aetiologies experienced referred sensations in different parts of their bodies during transcutaneous electric nerve stimulation, which was mainly applied to the ear. Most of the patients reported sensations in the painful regions, the most susceptible patients being those with phantom limb pain. Little description is given, apart from 3 patients with phantom pain who reported increased phantom sensations; 2 of the 3 experienced parts of their phantom limbs for the first time. It seems reasonable to suppose that in all such patients with major damage to the nervous system, grossly abnormal conditions pertain, and other than the unusual distribution of patterns of sensory referral, differentiation from Mitempfindungen is straightforward.

Further unusual patterns of cutaneous reference, different again from Mitempfindungen, have been described. These heterogeneous phenomena, which have been reported both in normals and in patients with various often poorly delineated neurological disease, are rare and ill-defined. Together with the extensive early literature, several of these conditions were considered at length by Ernest Jones (1907), and befittingly for Freud's biographer, he placed considerable emphasis on hysterical and other psychiatric conditions which he considered underlaid many of the published reports. Jones also considered in particular the first paper on the subject by Obersteiner (1881), who introduced the term allochiria. Obersteiner reported 4 patients with diseases of the nervous system in whom cutaneous stimuli were referred to the opposite side. The essential feature to which Obersteiner drew attention was that the patients were in doubt as to which side of the body had been touched, but neither intellectual nor sensory dysfunction was thought to account for the error. Jones rejected Obersteiner's use of the term allochiria, and called this condition dyschiria; he used the term synchiria for the very rare phenomenon of bilateral and probably strictly mirrored sensations that are evoked by unilateral stimulation. Synchiria may be allied to the phenomenon of congenital mirror movements (Schott and Wyke, 1981) with which it can coexist (Drinkwater, 1914).

The nosology of these conditions unfortunately remains confused. Thus Kawamura *et al.* (1987), in a recent study, used the word alloaesthesia to mean that a sensory stimulus given on one side of the body is perceived at the corresponding area on the other side. Yet in describing a number of patients sustaining cerebrovascular accidents or spinal cord lesions, at least one of the patients showed patterns of misreference that were ipsilateral. Moreover, Obersteiner (1881), whom these authors report as first describing the phenomenon (although calling it allochiria), specifically excluded any element of confusion in the patients, yet anosognosia is reported in a number of their patients, as is impairment of consciousness. Regardless of terminology, the cases of misreference described by Kawamura and coworkers (1987) have more in common with those described by Nathan (1956) and others referred to above, and with those patients with defective localization due to parietal lobe dysfunction (Critchley, 1953), and demonstrate the differences between Mitempfindungen and these other forms of cutaneous misreference.

The mechanisms underlying Mitempfindungen remain a matter for speculation. Concerning the nature of the stimulus, that this is innocuous and yet gives rise to a different and usually uncomfortable or even painful response, is reminiscent of trigeminal and similar neuralgias, in which painless trigger factors generate a painful response although in the same or nearby area. While in trigeminal neuralgia peripheral and central factors have been the subject of debate (Kerr, 1970), in Mitempfindungen central processing must be important, since ephaptic and other localized mechanisms could not explain the far distant sensory phenomena experienced.

Whether the site of stimulus production has specific properties is unclear. Since there is often a tiny skin papule or other localized cutaneous lesion at the point of stimulation, it may be that these trigger areas include the punctate cutaneous excrescences originally called Haarscheiben (*see* Richter, 1977). These structures consist of Merkel cells that surround the expanded, unencapsulated, slowly adapting type I mechanoreceptor endings of myelinated axons (Iggo, 1977). These units are particularly sensitive to edge contours indenting the skin (Johansson and Vallbo, 1983), and thus might be activated by firm scratching, which is the typical stimulus inducing Mitempfindungen. Such a possibility, however, does not take into account the cutaneous distribution of such units, nor the other mechanical methods such as irritation of a hair follicle by which Mitempfindungen can be induced. The occasional exacerbation of Mitempfindungen by minor skin infections or warmth suggests a lowered threshold in these circumstances, and the report of stimulation of areas around the trigger point abolishing Mitempfindungen (Mittelmann, 1920) raises the possibility of surround inhibition. It is clear, therefore, that both the histological structures and physiological mechanisms involved at the site of stimulation (and reference) remain unknown.

Concerning possible central pathways subserving Mitempfindungen, a number of possibilities which have been envisaged in other types of referred pain are untenable in view of the distances involved between the trigger and referral points. Thus mechanisms implicating the peripheral nerve and its branches (e.g., Sinclair *et al.*, 1948; Bahr *et al.*, 1981) seem unlikely, and equally improbable are localized spinal convergence mechanisms at segmental level, such as have been considered in referred visceral pain (for review, *see* Cervero and Morrison, 1986), in pain induced experimentally (Hockaday and Whitty, 1967) and in certain instances of causalgia (Schott, 1986). The topographical stability and stereotyped characteristics of an individual's Mitempfindungen do not suggest major central modulation; hence polysynaptic pathways which are perhaps more susceptible to such modulation are less likely to be involved. The pattern of ipsilateral referral and the punctate stimulus and Mitempfindungen are difficult to relate to any known functions or perturbations of the major spinal cord tracts, thalamus or cortical pathways. It may be, therefore, that congenital central neuronal branching, duplication or other such focal anatomical substrate, or an unusual specific pathway, could provide an explanation. What requires particular explanation is why only some individuals experience the phenomenon, and then repeatedly over years.

One pathway that could underlie Mitempfindungen is the spinocervical tract (for reviews, *see* Willis, 1985; Besson and Chaouch, 1987). The cells of origin of this tract lie in the dorsal horn, and the axons run in the ipsilateral dorsolateral quadrant and terminate at the lateral cervical nucleus at C1 and 2. Axons from this nucleus ascend and cross, joining the medial lemniscus and projecting to the thalamus and midbrain. Spinocervical tract cells are typically excited by mechanical stimuli such as from hair follicles, but the cells can also be activated by noxious stimuli and have input from A-alpha and beta, A-delta and C fibres, features which would be compatible with a pathway implicated in the mediation of Mitempfindungen. In particular, the very fact that Mitempfindungen are experienced only by certain people would indeed be consistent with the evident inconstancy of this pathway in humans: thus Truex *et al.* (1970) found the lateral cervical nucleus in 16 autopsies to be well developed in only 2, poorly developed in 7 and absent in 7. Clearly, however, the role of the spinocervical tract, as of any other spinal pathway, and the involvement of the thalamus, in particular the ventral posterior lateral nucleus, remains unknown. Why the 4 patients described here experienced transient Mitempfindungen is also unknown. It is possible that central neuronal damage, either direct or secondary to peripheral nerve damage (Wall, 1983), results in an area of focal hyperexcitability or reduced inhibition in an otherwise quiescent spinal pathway or thalamic nucleus.

Mitempfindungen represent a clinical curiosity, and the patients described here illustrate that the same or a closely related phenomenon can occur as an acquired condition. There are various other forms of reference of cutaneous sensation, although Mitempfindungen have characteristic and easily recognizable features. Mechanisms remain speculative and study of the phenomenon is frustrated by the evanescent nature of the subjective phenomenon, the lack of objective signs and indeed the observation in some people that the phenomenon tantalizingly tends to occur when the subject's attention is elsewhere.

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AUTONOMIC DISTURBANCES IN CLUSTER HEADACHE

by PETER D. DRUMMOND

(From the Department of Neurology, Prince Henry Hospital, and the School of Medicine, University of New South Wales, Australia)

SUMMARY

Ocular sympathetic function, facial flushing and the presence or absence of lachrymation and rhinorrhoea were examined in 30 patients during spontaneous or nitroglycerin-induced cluster headache. In 27 cases measurements were also obtained during the headache-free interval. Ocular sympathetic function was impaired on the symptomatic side between cluster attacks and function was reduced further during cluster headache. Greater heat loss from the orbital region on the symptomatic side was associated with ocular sympathetic dysfunction both during and between attacks, and with lachrymation during attacks. Heat loss from the cheek and side of the nose was greater on the symptomatic side in patients whose attack was accompanied by lachrymation, but heat loss from these areas was unrelated to the extent of ocular sympathetic deficit. These findings suggest that parasympathetic overactivity in the greater superficial petrosal nerve provokes facial flushing and lachrymation. Parasympathetic overactivity could also cause dilatation of the internal carotid artery and compression of the periarterial plexus of sympathetic fibres, producing a sympathetic deficit with release of vasoconstrictor tone in the eye. Thus autonomic disturbances in cluster headache may be explained by the unitary hypothesis of parasympathetic hyperactivity being responsible for ocular sympathetic deficit.

INTRODUCTION

An increase in blood flow in and around the affected eye during cluster headache has been well documented. For example, increases in corneal temperature, intraocular pressure and corneal indentation pulse amplitude indicate that intraocular blood flow increases during cluster headache (Hørven and Sjaastad, 1977). In 11 patients examined thermographically during spontaneous attacks of cluster headache, heat loss from the affected orbit and cheek was found to be greater than heat loss from the opposite side by up to 1.5 °C (Drummond and Lance, 1984). In another 22 patients examined during cluster attacks induced by nitroglycerin or alcohol, the increase in heat loss spread below the eye, down the side of the nose and to the temple on the symptomatic side. Other signs of unilateral extracranial vasodilatation during cluster headache include increases in extracranial blood flow, measured by rate of clearance of radioactive xenon (Sakai and Meyer, 1978), increases in temporal pulse

Correspondence to: Dr P. D. Drummond, Psychology Section, Murdoch University, Murdoch 6150, Western Australia.

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amplitude (Drummond and Anthony, 1985) and increases in velocity of blood flow through the supratrochlear artery on the symptomatic side (Schroth *et al.*, 1983).

The mechanism of the unilateral increase in extracranial blood flow in cluster headache is not clear. Drummond and Lance (1984) observed that the onset of pain usually preceded facial flushing in affected areas, and suggested that increases in blood flow were mediated by antidromic release of vasodilator substances from trigeminal nerve terminals, or reflexly by vasodilator fibres in the greater superficial petrosal (GSP) nerve activated by discharge of trigeminal pain pathways. Other mechanisms of facial flushing that do not appear to be relevant to the vascular changes of cluster headache include release of sympathetic vasoconstrictor tone in areas such as the ears and lips (Fox *et al.*, 1962) and active sympathetic vasodilatation in areas such as the forehead (Fox *et al.*, 1962; Drummond and Lance, 1987).

Autonomic symptoms and signs in cluster headache have been attributed to parasympathetic discharge (Gardner *et al.*, 1947), sympathetic paralysis (Fanciullacci *et al.*, 1982), or a combination of both through some unspecified central integrative mechanism (Spierings, 1980). Increases in lachrymation and nasal secretion during cluster headache (Saunte, 1984) indicate that parasympathetic activity increases in the GSP nerve. The presence of lachrymation during cluster headache was found to correlate with the extent of heat loss from periorbital regions on the symptomatic side (Drummond and Lance, 1985), thus suggesting that facial flushing as well as lachrymation was mediated by the GSP nerve.

Transitory and permanent ocular sympathetic deficits have been observed in a minority of patients with cluster headache. Of 90 patients studied by Kunkle and Anderson (1960), miosis (sometimes accompanied by ptosis) was permanent in 7 patients and was noted only during cluster attacks in another 7 cases. Nieman and Hurwitz (1961) reported that ocular sympathetic deficits were permanent in 10 of 50 cluster headache patients while miosis and ptosis were transitory in 1 other patient. Pharmacological investigation of cluster headache patients with permanent ocular sympathetic deficits indicated that the lesion was postganglionic in such cases (Riley and Moyer, 1971; Vijayan and Watson, 1982). Fanciullacci *et al.* (1982) investigated ocular sympathetic function in 45 cluster headache patients, including 5 with permanent miosis on the symptomatic side. They reported that the pupil on the symptomatic side invariably dilated less than the opposite pupil in response to installation of tyramine eyedrops in 26 patients examined between attacks and in a further 19 patients examined between bouts. Since tyramine releases stores of noradrenaline from sympathetic nerve terminals, diminished pupillary dilatation can be taken as evidence of a postganglionic lesion in the ocular sympathetic pathway. Fanciullacci *et al.* (1982) concluded that a unilateral ocular sympathetic deficit persisted subclinically in all cluster headache patients, and suggested that this deficit may be of primary importance in the precipitation and localization of cluster attacks. For example, release of sympathetic vasoconstrictor tone during cluster headache could cause painful extracranial vasodilatation, although the lack of relationship between the presence or absence of ptosis and asymmetry of heat loss from various facial areas during cluster headache does not support this view (Drummond and Lance, 1985). Moreover, sympathetic block does not cause pain or precipitate headache in human subjects.

Sympathetic denervation of the iris causes the pupil to constrict more rapidly to light and to dilate more slowly in darkness than the normally innervated pupil (Lowenstein and Loewenfeld, 1950*a*, *b*). In the present investigation, speed of pupillary dilatation and constriction were used as indices of ocular sympathetic function in patients with cluster headache. Since muscle tone of the eyelids is mediated by the sympathetic nervous system (Walsh and Hoyt, 1969), the width of the palpebral fissures was also measured.

To clarify the mechanism of extracranial vasodilatation in cluster headache, the relationship between facial flushing (measured thermographically), ocular sympathetic deficits and signs of GSP nerve activity (lachrymation and rhinorrhoea) was investigated. Measures were taken during and between attacks of cluster headache.

METHOD

Patients

Twenty-nine men and 1 woman who met the criteria for cluster headache (Ad Hoc Committee, 1962) (Table 1) were studied during a spontaneous attack (9 patients) or an attack induced by nitroglycerin (21 patients). In 27 patients, measurements were also obtained before or after the attack. Patients' ages ranged from 26 to 58 yrs (average age 41 yrs). Informed consent for the procedures was obtained in each case.

Procedure

Except for 9 patients who arrived at the laboratory with a spontaneous attack of cluster headache, thermographic and pupillary measurements were first carried out between attacks at least 20 min after

TABLE 1. CLINICAL CHARACTERISTICS OF THE 30 CLUSTER HEADACHE PATIENTS

	No. of patients (total = 30)
Classification	
Initial bout	3
Episodic cluster	24
Chronic cluster	3
Pain	
Unilateral	30
One or more attacks per day	28
Severe within 20 min	28
Duration usually less than 3 h	27
Associated ipsilateral signs	
Lachrymation	24
Rhinorrhoea	25
Ptosis	21
One or more of the above	28

the patient arrived so that he or she could adjust to the lighting and room temperature of 22 ± 1 °C. Measurements were repeated and the presence or absence of lachrymation and rhinorrhoea was evaluated clinically at the height of cluster headache developing 10 to 90 min after sublingual administration of 0.9 mg of nitroglycerin. In 6 patients who had presented with spontaneous cluster headache, measurements were repeated 30 to 60 min after the attack had subsided.

Thermograms were taken with an AGA Thermovision 680 camera which detected 7 consecutive isotherms, each separated by 0.5 °C. Because facial temperature may have been influenced by the pupillographic procedure, asymmetry of heat loss instead of absolute facial temperature was measured to the nearest 0.25 °C from the supraorbital region (lower one-third of the forehead), orbits, cheeks and nose.

Photographs of both pupils were taken at intervals of 30 s on infrared film via an infrared flash and camera of fixed focal length, housed behind a two-way mirror in a small light-tight photographic chamber. Pupil diameter in darkness (0.04 Lux) was recorded in all 30 patients. In 23 cases, the pupils were photographed after the chamber had been illuminated for 0.8, 1.6 or 3.0 s with moderately bright light (153.5 Lux, measured with a Manolux electronic Luxmeter at the position of the patient's eyes in the photographic chamber). After the chamber had been illuminated for 30 s, photographs were taken 3.0 or 5.0 s after the light had been switched off (Loewenfeld and Rosskothen, 1974). Subjects were instructed to look at a black dot 56 cm away attached to an opaque screen shielding the light source, and to stop blinking for a few seconds before each photograph. Two series of photographs were taken during the headache-free interval and a further series was taken at the height of the attack.

Horizontal pupil diameter and the width of the palpebral fissures (maximum vertical distance betwen the eyelids) were later measured with calipers from the film negative magnified × 8.0. To exclude the possibility that photophobia caused blepharospasm, the width of the palpebral fissures was measured in darkness.

RESULTS

Because preliminary analyses indicated that spontaneous and nitroglycerin-induced attacks were indistinguishable on the measures recorded, combined results are presented.

Thermography

Between attacks, heat loss for the group as a whole was symmetric in all regions of the face (Table 2). At the height of the cluster attack, heat loss from the eye, cheek and nose was greater on the affected side (Table 2).

TABLE 2. DIFFERENCE IN HEAT LOSS BETWEEN THE SYMPTOMATIC AND NONSYMPTOMATIC SIDES OF THE FACE IN CLUSTER HEADACHE (MEAN±SD)

	Thermographic asymmetry (°C)	
Site	*Between attacks (n = 27)*	*During attack (n = 30)*
Supraorbital region	−0.04 ± 0.27	−0.08 ± 0.44
Orbits	0.04 ± 0.21	0.38 ± 0.42**
Cheeks	−0.01 ± 0.27	0.21 ± 0.45**
Nose*	−0.03 ± 0.21	0.21 ± 0.47**

* Temperature of the nose was out of range of the thermographic apparatus in 2 cases. ** Heat loss significantly greater on the symptomatic side ($P < 0.05$).

Pupillography

Both during and between attacks, the pupil on the symptomatic side constricted more rapidly to light and dilated more slowly in darkness than the opposite pupil (figs 1, 2). Diameter of the symptomatic pupil after 5.0 s of darkness was at least 0.5 mm smaller than the opposite pupil in 6 of 21 patients during the headache-free interval, and in 6 of 23 cases during cluster headache (Table 3).

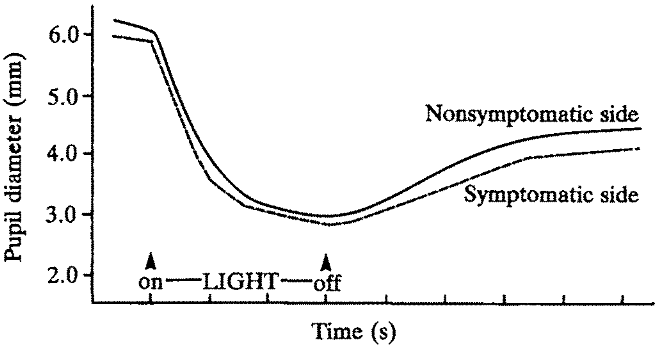

FIG. 1. Average response of the symptomatic and nonsymptomatic pupils to 3.0 s of moderately bright light in 23 patients with cluster headache, demonstrating dilatation lag on the painful side. Continuous line = nonsymptomatic side, broken line = symptomatic side.

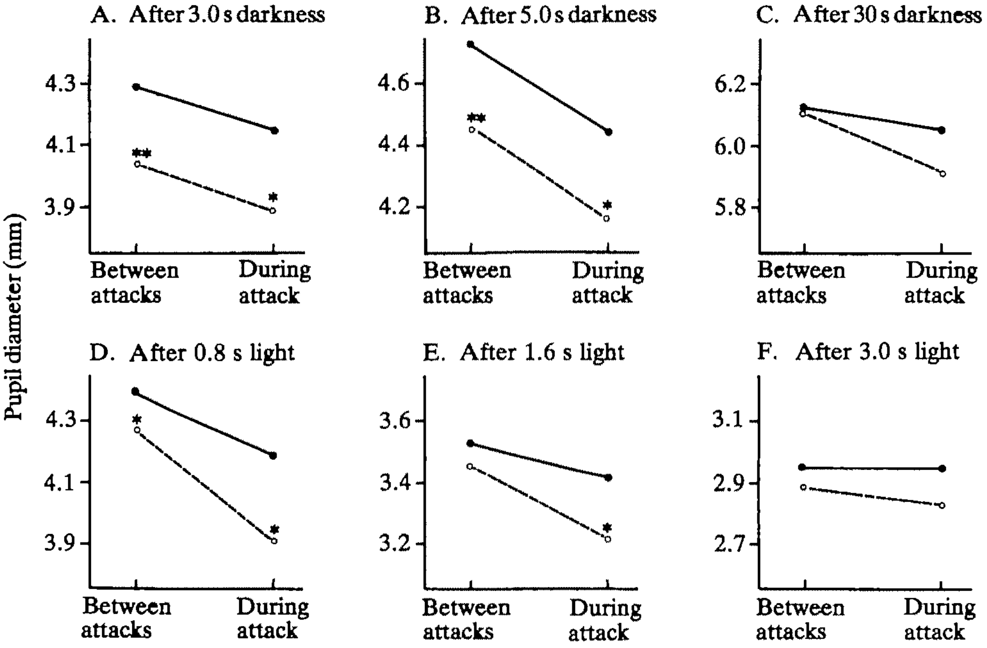

FIG. 2. Pupillary light reflex during (n = 23) and between (n = 21) attacks of cluster headache. Diameter of symptomatic pupil significantly smaller than diameter of nonsymptomatic pupil: * $P < 0.05$, ** $P < 0.01$. Continuous line = nonsymptomatic side; broken line = symptomatic side.

TABLE 3 EXTENT OF OCULAR SYMPATHETIC DYSFUNCTION ON THE SYMPTOMATIC SIDE DURING AND BETWEEN ATTACKS OF CLUSTER HEADACHE

	Dilatation lag		*Ptosis*	
	Between ($n = 21$)	*During* ($n = 23$)	*Between* ($n = 26$)	*During* ($n = 30$)
Absent	7	10	11	9
Less than 0.5 mm	8	7	8	6
0 5 mm or more	6	6	7	15

After 30 s of darkness, pupil diameter did not differ significantly between the symptomatic and nonsymptomatic sides (fig. 2C). The speed of dilatation of both pupils in darkness was greater during the headache-free interval than during an attack (after 5.0 s of darkness, F(1,20) = 6.93, $P < 0.05$, *see* fig. 2B). Both pupils also constricted more rapidly to light during cluster headache than during the headache-free interval, particularly on the symptomatic side. After 0.8 s of light, the decrease in average pupil diameter was greater during than between attacks (F(1,20) = 4.45, $P < 0.05$, *see* fig. 2D); after 1.6 s of light, the symptomatic pupil had constricted more than the opposite pupil during headache but not during the headache-free interval (interaction between presence or absence of headache and pupil asymmetry, F(1,20) = 6.52, $P < 0.05$, *see* fig. 2E).

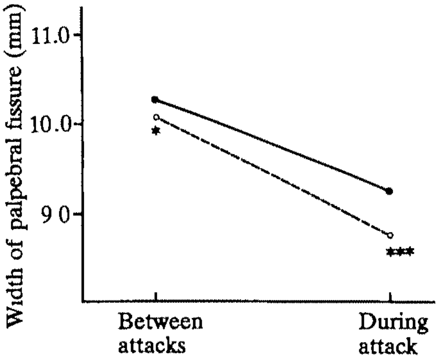

FIG 3. Width of the palpebral fissures during (n = 30) and between (n = 26) attacks of cluster headache. Significant reduction on the symptomatic side: * $P < 0.05$; *** $P < 0.001$ Continuous line = nonsymptomatic side; broken line = symptomatic side.

Both during and between attacks, the palpebral fissure was narrower on the symptomatic side (fig. 3). Asymmetry was greatest during cluster attacks (interaction between presence or absence of headache and asymmetry of the palpebral fissures, F(1,25) = 7.08, $P < 0.05$). Ptosis of at least 0.5 mm on the symptomatic side was observed in 7 of 26 patients during the headache-free interval and in 15 of 30 patients during cluster headache (Table 3).

Relationships among autonomic changes

Between attacks, pupil asymmetry after 5.0 s of darkness and 1.6 s of light was greatest in patients showing increased heat loss from the orbital region on the symptomatic side (Table 4). This relationship persisted during cluster headache (Table 5). Ptosis on the symptomatic side during cluster attacks was greatest in patients with pupillary dilatation lag but was unrelated to thermographic asymmetry (Table 5).

Greater heat loss from the orbit, cheek and nose on the symptomatic side was observed most frequently in patients showing lachrymation during the cluster attack (Table 6). In such patients, the symptomatic pupil dilated more slowly in darkness and constricted more rapidly to light than the opposite pupil (Table 6). Ptosis on

TABLE 4 CORRELATION BETWEEN THERMOGRAPHIC ASYMMETRY AND ASYMMETRY OF OCULAR SYMPATHETIC FUNCTION IN THE HEADACHE-FREE INTERVAL

	Thermographic asymmetry				*Pupil asymmetry*		
	Supraorbital	*Orbits*	*Cheeks*	*Nose*	*Constriction*^{a}	*Dilatation lag*^{b}	*Ptosis*^{c}
Thermographic asymmetry							
Supraorbital	—						
Orbits	0.03	—					
Cheeks	0.25	0.26	—				
Nose	0.25	−0.18	0.29	—			
Pupil asymmetry							
Constriction^a	−0.12	0.54*	−0.12	−0.03	—		
Dilatation lag^b	−0.11	0.49*	−0.05	0.09	0.85*	—	
Ptosis^c	−0.03	−0.24	−0.13	0.19	0.18	0.31	—

* $P < 0.05$. ^{a} Difference in pupil diameter between the nonsymptomatic and symptomatic sides after exposure to light for 1.6 s. ^{b} Difference in pupil diameter between the nonsymptomatic and symptomatic sides after 5.0 s of darkness. ^{c} Difference in width of the palpebral fissures between the nonsymptomatic and symptomatic sides.

TABLE 5 CORRELATION BETWEEN THERMOGRAPHIC ASYMMETRY AND ASYMMETRY OF OCULAR SYMPATHETIC FUNCTION DURING CLUSTER HEADACHE

	Thermographic asymmetry				*Pupil asymmetry*		
	Supraorbital	*Orbits*	*Cheeks*	*Nose*	*Constriction*^{a}	*Dilatation lag*^{b}	*Ptosis*^{c}
Thermographic asymmetry							
Supraorbital	—						
Orbits	0.30	—					
Cheeks	0.52*	0.64*	—				
Nose	0.57*	0.53*	0.77*	—			
Pupil asymmetry							
Constriction^a	−0.11	0.59*	0.30	0.37	—		
Dilatation lag^b	−0.26	0.59*	0.21	0.30	0.87*	—	
Ptosis^c	0.13	0.32	0.30	0.23	0.27	0.54*	—

* $P < 0.05$. ^{a} Difference in pupil diameter between the nonsymptomatic and symptomatic sides after exposure to light for 1.6 s. ^{b} Difference in pupil diameter between the nonsymptomatic and symptomatic sides after 5.0 s of darkness. ^{c} Difference in width of the palpebral fissures between the nonsymptomatic and symptomatic sides.

TABLE 6 ASYMMETRY OF HEAT LOSS AND OCULAR SYMPATHETIC FUNCTION (MEAN±SD) IN RELATION TO PRESENCE OR ABSENCE OF LACHRYMATION DURING CLUSTER HEADACHE

	Lachrymation			
	Present (*n* = *12*)	*Absent* (*n* = *18*)	*t test*[a]	*P*
Thermographic asymmetry (°C)				
Supraorbital region	−0.04±0.44	−0.10±0.45	0.33	—
Orbits	0.58±0.19*	0.25±0.48*	2.28	< 0.05
Cheeks	0.42±0.50*	0.07±0.36	2.20	< 0.05
Nose	0.46±0.49*	0.03±0.39	2.59	< 0.05
Pupil asymmetry (mm)[b]				
Light on for 1.6 s	0.4±0.4*	0.1±0.3	2.08	< 0.05
Light off for 5.0 s	0.6±0.6*	0.1±0.5	2.51	< 0.05
Ptosis (mm)	0.7±0.6*	0.3±0.6*	1.65	—

[a] Statistical comparison of groups with and without lachrymation. [b] Lachrymation was present in 10 patients and absent in 13 others * Heat loss significantly greater, pupil significantly smaller or significant ptosis on symptomatic side ($P < 0.05$)

the symptomatic side was unrelated to the presence or absence of lachrymation (Table 6).

Rhinorrhoea was observed in 8 of 30 patients at the height of cluster headache. Heat loss from the affected side of the nose was greater in this subgroup; the difference between sides averaged 0.47 °C ($P < 0.05$) compared with 0.11 °C (not statistically significant) in patients without rhinorrhoea (comparison between groups, $t(26) = 1.87$, $P < 0.10$). No other relationship between rhinorrhoea and thermographic or pupillary measures was observed.

DISCUSSION

During cluster attacks heat loss from the affected eye, cheek and side of the nose was greater than from corresponding regions on the nonsymptomatic side of the face. The development of this flush has been attributed previously to a trigeminal-GSP nerve vasodilator reflex or to release of vasoactive substances from trigeminal nerve terminals (Drummond and Lance, 1984).

A decrease in ocular sympathetic function on the symptomatic side was detected between cluster attacks. In particular, the symptomatic pupil dilated more slowly in darkness and constricted more rapidly to light than the opposite pupil. Ptosis was also present on the symptomatic side. The deficit in ocular sympathetic function was greatest in patients demonstrating increased heat loss from the orbital region on the symptomatic side even between headaches, thus suggesting a persisting release of sympathetic vasoconstrictor tone in conjunctival vessels. In contrast to the orbit, heat loss from other regions of the face was unrelated to indices of pupillary sympathetic function.

During cluster attacks, ptosis increased and the pupil on the symptomatic side constricted more rapidly to light than during the headache-free interval, indicating additional release of ocular sympathetic tone. Both pupils dilated more slowly in darkness and constricted more rapidly to light during cluster attacks than during the headache-free interval. In all but 2 cases the first measurement, when the novelty of the pupillographic procedure would be expected to cause most activation of the sympathetic nervous system, was carried out between attacks. Thus the bilateral decrease in ocular sympathetic activity during cluster headache may have been due, at least in part, to the order in which measurements were taken.

The relationship between lachrymation and unilateral facial flushing during cluster headache confirmed previous findings (Drummond and Lance, 1985). An additional association between lachrymation and ocular sympathetic deficit on the symptomatic side was observed in the present study. Our previous findings indicated that an increase in parasympathetic activity in the GSP nerve could be responsible for the simultaneous occurrence of lachrymation and facial flushing (Drummond and Lance, 1984, 1985). A vasodilator reflex, initiated by stimulation of the trigeminal nerve and brainstem nuclei such as the locus coeruleus (Gonzalez *et al.*, 1975) and mediated by the sphenopalatine and otic ganglia (Lambert *et al.*, 1984), has been found to increase facial temperature and flow through the common carotid artery in cats. Dilatation of the internal carotid artery or swelling of its arterial wall during cluster headache could possibly be mediated by the trigeminal-GSP nerve vasodilator reflex since parasympathetic fibres pass to the internal carotid siphon via the deep petrosal nerve (Chorobski and Penfield, 1932) and via the GSP nerve and sphenopalatine ganglion (Walters *et al.*, 1986). In this case ocular sympathetic deficits could be caused by compression of the plexus of sympathetic fibres surrounding the internal carotid artery. If this is the explanation, the degree of sympathetic deficit should correlate with indicators of GSP hyperactivity such as lachrymation. This was so for pupillary constriction and dilatation lag but not for ptosis (Table 6).

Differences in distribution of sympathetic vasoconstrictor fibres to various parts of the face (Fox *et al.*, 1962) may explain why ocular sympathetic dysfunction was associated with increased heat loss from the symptomatic orbit but not elsewhere. Thus facial flushing in cluster headache may be caused by the cumulative effects of trigeminal-GSP nerve hyperactivity and loss of sympathetic vasoconstrictor tone, the contribution of each mechanism varying in different facial areas.

Sjaastad and Fredriksen (1986) postulated recently that autonomic phenomena and pain are activated independently during cluster headache. Although the present findings do not exclude this possibility, it seems likely that certain autonomic disturbances are maintained or exacerbated by trigeminal-GSP nerve reflexes after pain develops. Further studies of the time course of autonomic changes and pain in cluster headache are required to clarify this issue.

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CHOREA AND MYOCLONUS IN THE MONKEY INDUCED BY GAMMA-AMINOBUTYRIC ACID ANTAGONISM IN THE LENTIFORM COMPLEX

THE SITE OF DRUG ACTION AND A HYPOTHESIS FOR THE NEURAL MECHANISMS OF CHOREA

by A. R. CROSSMAN, I. J. MITCHELL, M. A. SAMBROOK *and* A. JACKSON

(*From the Department of Cell and Structural Biology, School of Biological Sciences, University of Manchester, Manchester*)

SUMMARY

Experiments are described in which the gamma-aminobutyric acid (GABA) antagonist bicuculline was injected into the lentiform complex of conscious monkeys. Injections into either the lateral segment of the globus pallidus, or the medial part of the putamen, gave rise to chorea of the contralateral limbs and/or orofacial region. Control injections of vehicle alone were without effect. Injections of bicuculline into the lateral part of the putamen gave rise to contralateral myoclonus. The chorea produced by lateral pallidal or medial putaminal injections was virtually indistinguishable from the dyskinesia (chorea/ballism) which has been shown, in previous studies, to be induced by injection of GABA antagonists into the subthalamic nucleus.

It is proposed that the primary site of action of the GABA antagonist in producing chorea, in the present studies, was the lateral segment of the globus pallidus. The mode of action is suggested to be interruption of GABAergic transmission from the striatum to the lateral pallidal segment. Since this also occurs in Huntington's disease, it is proposed that experimental chorea induced by this method in the monkey may be a useful model of the dyskinesia seen in Huntington's disease in man. Loss of influence of inhibitory striatopallidal fibres would lead to abnormally increased activity of lateral pallidal neurons. These in turn project to the subthalamic nucleus, upon which they have an inhibitory action. Dyskinesia is thus produced by physiological inhibition of the subthalamic nucleus, whose destruction, both in man and the monkey, is known to produce ballism. It is proposed that ballism and chorea share common neural mechanisms, both involving the loss of influence of the subthalamic nucleus on the medial segment of the globus pallidus.

INTRODUCTION

Chorea in man

Chorea is the generic term used to describe patterns of abnormal involuntary movements having certain characteristic features. The archetypal form occurs in Huntington's disease, a progressive neurodegenerative condition which is inherited

Correspondence to: Dr A. R. Crossman, Experimental Neurology, Department of Cell and Structural Biology, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

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in an autosomal dominant fashion. Chorea may also be observed in a variety of other conditions including L-DOPA-induced dyskinesia, hemiballism and tardive dyskinesia.

It is difficult to provide a succinct definition of chorea, but most authorities seem to be in general agreement as to the physical appearance, nature, distribution and range of dyskinetic phenomena that may be termed choreic or choreiform. Chorea has been defined as 'a state of excessive, spontaneous movements, irregularly timed, nonrepetitive, randomly distributed and abrupt in character' (Classification of Extrapyramidal Disorders, 1981). Despite the apparently haphazard nature of the movements they have been likened to isolated fragments of normal voluntary movements, and attention has been drawn to certain sequences or patterns which are particularly characteristic. These include 'opening of the hand from a fully extended pronated posture with pronation of the wrist to closure with a trapped thumb and supinated wrist' (Bruyn, 1968). Another is 'a downward sweep of the chin with pursing of the lips followed by extension of the neck with labial retraction' (Bruyn, 1968).

Neuropathological changes in Huntington's disease centre on the basal ganglia and cerebral cortex (Bruyn, 1968). Within the basal ganglia the neostriatum bears the brunt of the disease, becoming severely atrophic with loss of striatopallidal and striatonigral fibre systems (Bruyn, 1968; Gebbink, 1968). Amongst the numerous neurochemical changes found in Huntington's brains are decreases in gamma-aminobutyric acid (GABA), its synthesizing enzyme glutamic acid decarboxylase (GAD), choline acetyltransferase (ChAT) and substance P in the neostriatum (McGeer *et al.*, 1973; Perry *et al.*, 1973; Bird and Iversen, 1974; McGeer and McGeer, 1976; Gale *et al.*, 1978). In addition, decreased GAD activity, and substance P and met-enkephalin-like immunoreactivity, have been demonstrated in the globus pallidus and substantia nigra (Bird *et al.*, 1973; McGeer *et al.*, 1973; Emson *et al.*, 1980). The loss of markers for GABA and neuropeptides from the globus pallidus and substantia nigra probably reflects the loss of GABAergic and peptidergic striatopallidal and striatonigral projection neurons. The nigrostriatal dopaminergic projection appears to be relatively spared (Bird and Iversen, 1974).

Primate models of chorea

A realistic primate model of human chorea has proved difficult to achieve. No naturally-occurring primate equivalent of Huntington's disease is known, and ablative lesions of the striatum do not produce choreiform movements (Wilson, 1914; Mettler, 1942). A number of experimental models have been described in subprimates, such as that produced by injection of the excitotoxin kainic acid into the neostriatum of the rat (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Coyle *et al.*, 1978; McGeer *et al.*, 1979). This reproduces some of the morphological and biochemical characteristics of the human disease, including loss of nerve cells from the striatum, with depletion of GABAergic and cholinergic indices. There is also loss of GABA and GAD from the substantia nigra, associated with the degeneration of striatonigral projection neurons. Surprisingly, the possibility of developing a primate model of Huntington's disease by the intrastriatal administration of excitotoxins does not appear to have been examined systematically. Kanazawa *et al.* (1986) have, however, reported that 'choreic' movements can be provoked by L-DOPA administration to monkeys following a kainic acid-induced lesion of the striatum.

Choreiform movements have, additionally, been described in monkeys as part of the dyskinetic states induced by various other experimental manipulations. These include lesions of the subthalamic nucleus (Whittier and Mettler, 1949), injection of GABA antagonists into the subthalamic nucleus or corpus striatum (Crossman and Jackson, 1984; Crossman *et al.*, 1984; Jackson and Crossman, 1984), and injection of the cholinergic agonist carbachol into the putamen or head of the caudate nucleus (Murphey and Dill, 1972).

A model of choreiform movement, of particular clinical relevance, has recently been developed in primates in the form of L-DOPA-induced dyskinesia (Bédard *et al.*, 1986; Clarke *et al.*, 1987*a*, *b*; Crossman, 1987). This is reliably produced in monkeys, made parkinsonian by administration of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and subsequently treated for 4–8 weeks with therapeutic doses of L-DOPA.

Background to the present studies

In previous reports we have described the hemiballistic and choreiform dyskinesia which can be induced in the conscious monkey by injection of GABA antagonists into the subthalamic nucleus (Crossman *et al.*, 1980, 1981, 1984; Crossman, 1987). In these studies a large number of injections were made throughout the ventral diencephalon and globus pallidus in order to control for drug diffusion and thus permit confident identification of the site of action in inducing hyperkinesia. It was noted that injections into a small number of sites in the lateral segment of the globus pallidus (Crossman *et al.*, 1984) and adjacent putamen (Jackson and Crossman, 1984) induced choreoathetoid movements of the contralateral limbs. In view of these early experimental observations, and the likely importance of altered basal ganglia GABA function in the pathophysiology of human chorea, the present study was undertaken. Its aim was systematically to study the effects of regional GABA antagonism in the lentiform complex of the conscious monkey in order to (1) define more precisely the nature of the dyskinesia; (2) define the regions from which choreic movements could be provoked; and (3) shed light on the possible pathophysiological mechanisms of chorea.

METHODS

Experiments were performed on 14 adult monkeys (Macaca fascicularis, Macaca nemestrina, Papio papio or Papio cynocephalus) of either sex. The animals were anaesthetized with ketamine hydrochloride (15 mg/kg) and xylazine (3.0 mg/kg) administered intramuscularly, and supplemented as necessary. All surgical procedures were performed taking aseptic precautions. In order to improve stereotactic accuracy, approximately 1 ml Omnipaque contrast medium (Nyegaard) was injected stereotactically into the lateral ventricle and stereotactic ventriculography was performed, as described in detail elsewhere (Crossman *et al.*, 1984) This permitted visualization of intracerebral landmarks, including the anterior and posterior commissures, from which corrections were made to standard stereotactic atlases for the placement of intracerebral cannulae.

Implantation of cannula guides

Under general anaesthesia, stainless steel cannula guides were inserted into the brain, aimed at the lentiform complex, under stereotactic control. They were secured to the skull using stainless steel screws and dental acrylic cement. The cannula guides consisted of a guide tube (0.46 mm o.d., 0.25 mm i.d.) attached to a base, the latter being located on the surface of the skull to serve as anchorage for the cement. When not in use, the cannula guide contained a blank which bore a stainless steel stylette (0.23 mm diameter) to ensure patency of the guide tube (Needham *et al.*, 1983).

Intracerebral drug injections

In order to make intracerebral drug injections, the animal was lightly anaesthetized with ketamine, administered i.m. The central blank was removed from the cannula guide and replaced with a mechanically-operated micropump, which contained either a solution of bicuculline methiodide in sterile saline (15 μg/μl) or vehicle alone. The pump bore an injection needle (0.20 mm diameter) which passed through the guide tube and into the structure to be injected. The construction and operation of the pump have been described in detail elsewhere (Needham *et al.*, 1983). The animal was allowed to recover fully from anaesthesia, and the pump was then triggered to deliver a volume of 2 μl. Animals were observed for a minimum of 1 h after injection and any abnormalities of movement recorded on film or videotape.

Confirmation of injection sites

At the end of the experiments the animals were anaesthetized with ketamine and a lethal dose of barbiturate was then administered. Cannula tracks were marked by injection of 1 μl of India ink at the site of the deepest injection. The animals were perfused transcardially with 0.9% saline followed by 10% neutral phosphate-buffered formalin. Brains were blocked stereotactically and frozen sections were prepared at 20–40 μm. Selected series of sections were mounted and stained with cresyl violet for microscopic examination and reconstruction of injection sites.

RESULTS

The GABA antagonist bicuculline methiodide was injected on 152 occasions at 121 separate intracerebral sites in the conscious monkey. Postmortem histological study indicated that most injections were located in the lentiform complex (medial or lateral segments of the globus pallidus or the putamen). Abnormalities of movement (dyskinesias) were observed following injections at 64 sites. Thirty-four of these lay in the putamen, and 17 in the lateral segment of the globus pallidus. The Table summarizes the regional distribution of both effective and noneffective injection sites and the nature of the dyskinesia provoked. The precise locations of all individual injection sites are illustrated diagrammatically in the fig. The injection sites associated with dyskinesia are given in the Appendix, together with the nature of the dyskinesia elicited, the body parts involved, the severity of the movements and the latency to onset.

Two main dyskinetic phenomena were encountered in this study, depending upon

TABLE ANATOMICAL LOCATION OF EACH OF THE 121 SITES WHICH WERE INJECTED WITH BICUCULLINE METHIODIDE AND THE NUMBER OF SITES, IN EACH ANATOMICAL AREA, FROM WHICH DYSKINESIA WAS ELICITED

		No. of sites giving dyskinesia		
Structure	*No. of injection sites*	*Chorea*	*Myoclonus*	*Total*
Putamen	61	28	9	34
Globus pallidus (lateral segment)	23	16	0	17*
Substantia innominata	13	5	0	5
Amygdala	9	3	0	3
Anterior commissure/extreme capsule	6	0	0	0
Globus pallidus (medial segment)	3	2	0	2
Caudate nucleus	2	0	0	0
Corona radiata	2	0	2	2
Internal capsule	1	0	0	1*
Optic tract	1	0	0	0

* Injections at 1 site in the lateral segment of the globus pallidus (site 56) and 1 site in the internal capsule (site 55) gave rise to contralateral torticollis and circling only.

the precise site of drug injection. The most common dyskinesia observed was chorea, in which the limbs and/or orofacial musculature were involved in abnormal involuntary activity to varying degrees. Less commonly observed was myoclonus, which was usually, although not always, elicited following injections at sites separate from those giving chorea.

Chorea

Nature of the dyskinesia. Chorea always occurred contralateral to the side of injection. Dyskinesia of the lower limb often began with alternating internal and external rotations of the ankle joint. As the disorder progressed, flexion and extension of the ankle joint appeared. The more distal joints of the foot also usually became involved, displaying an alternating flexion and extension. Initially, these dyskinetic movements were masked by weight-bearing. As they became more pronounced, however, they were obvious even during weight-bearing and the animal came to favour the unaffected limb. In instances where the dyskinesia became more severe, it often spread to involve the more proximal musculature such that the knee exhibited alternating flexion/extension and there occurred circumductive movements of the hip. On some occasions the first sign of lower limb dyskinesia was, in fact, alternating internal/external rotation of the hip with the foot, if weight-bearing, held in one spot.

Dyskinesia of the upper limb typically commenced with intermittent periods of repetitive, alternating pronation and supination of the forearm. In its initial stage the dyskinesia was effectively masked by gripping, but with the passage of time the disorder progressed and increased in severity. The amplitude of the movements

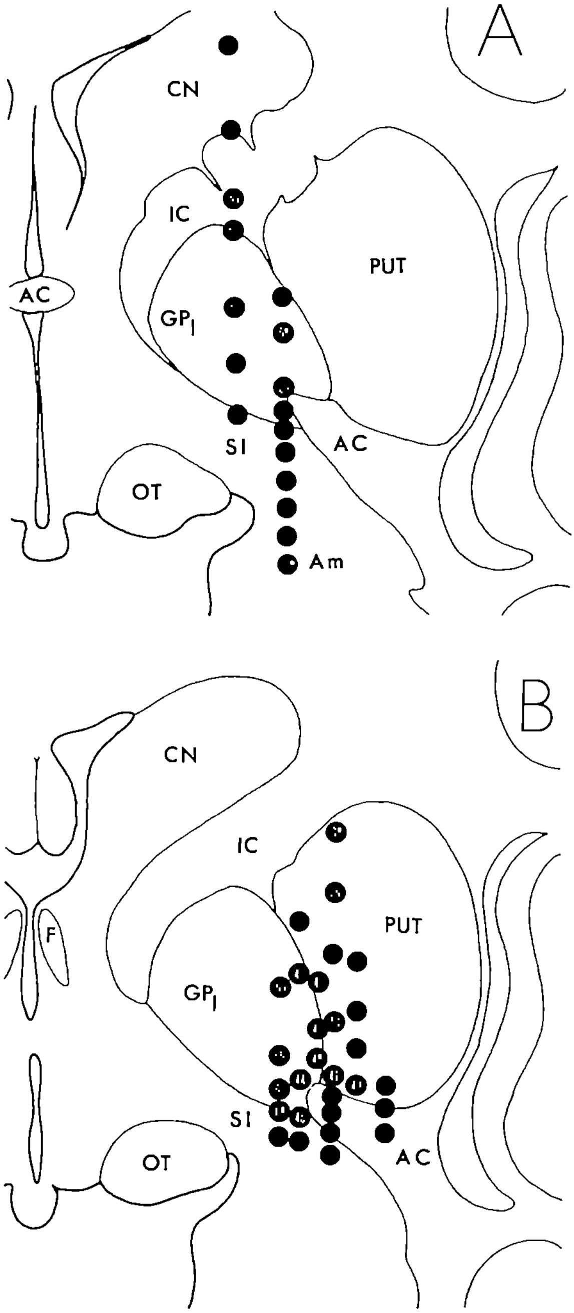
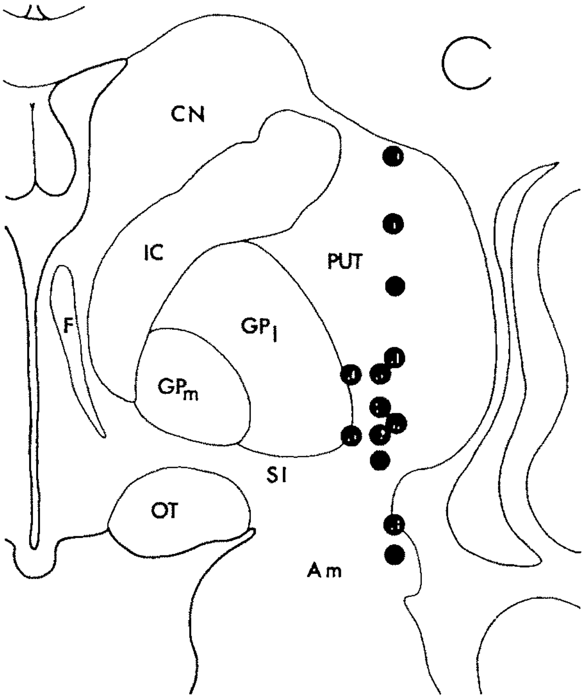


FIG. Semidiagrammatic representations of an anteroposterior series (A–F, continued on following pages) of coronal sections through the monkey brain. All of the individual sites at which injections of bicuculline were made are indicated by a black dot. Sites from which dyskinesia was provoked are identified numerically, and details of the characteristics of dyskinesia are given in the Appendix. All injection sites are represented on the right side, although injections were made, approximately equally, on both sides of the brain.

AC = anterior commissure; Am = amygdala; CN = caudate nucleus; F = fornix; GPl = globus pallidus (lateral segment); GPm = globus pallidus (medial segment); GPmo, GPmi = outer and inner divisions of GPm, respectively; IC = internal capsule; OP = optic tract; PUT = putamen; SI = substantia innominata.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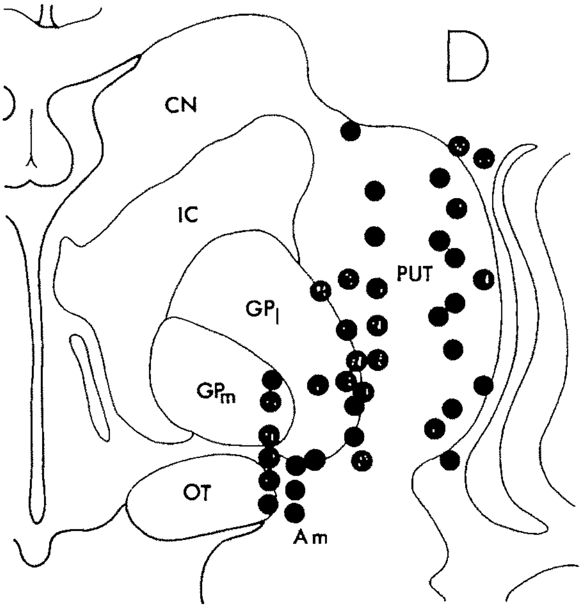
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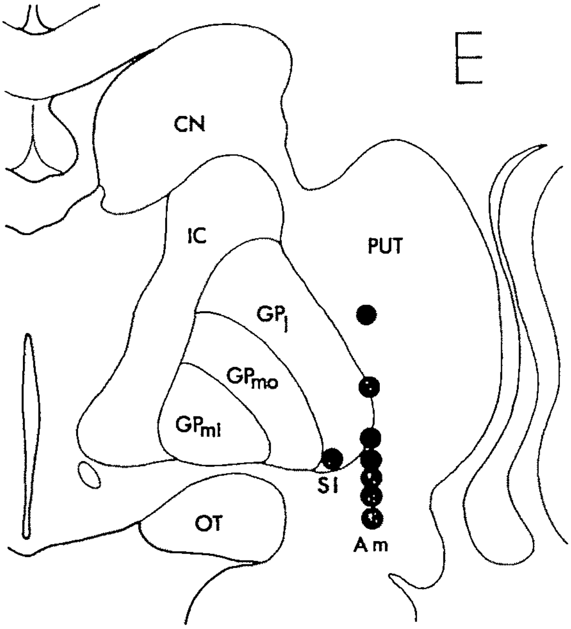
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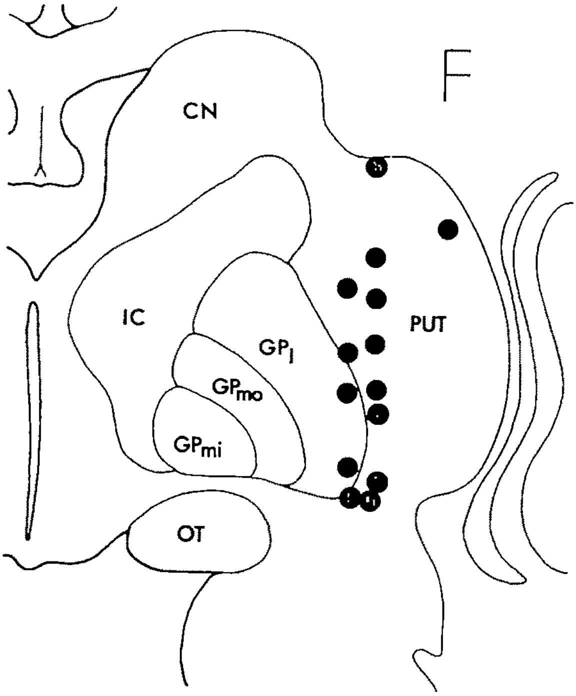
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became greater, and simultaneous movements about several joints began to occur. The distal musculature was almost invariably the first to be additionally involved, so that there developed a complex pattern of alternating flexion and extension of the fingers, flexion/extension of the wrist and pronation/supination of the forearm. If the limb was resting on the perch, for example, the movements gave the impression that the animal was trying to wipe something from the back of its hand. When the limb was used for weight-bearing on the perch, a common observation was that the metacarpophalangeal joints were hyperextended and, superimposed upon this, there occurred flexion/extension movements of the metacarpophalangeal joints and wrist. As with the lower limb, in instances where the dyskinesia became more marked, there gradually developed involvement of the more proximal musculature, characterized by flexion and extension of the elbow and circumductive movements of the shoulder joint. Comparison of film and videotape recordings indicated that the dyskinesia was virtually indistinguishable from that which we have described, in previous reports (Crossman *et al.*, 1980, 1981, 1984), to be produced by injection of GABA antagonists into the subthalamic nucleus.

Dyskinesia involving the orofacial musculature was encountered on a number of occasions, most often accompanied by more generalized chorea, but sometimes in isolation. It usually took the form of persistent chewing movements, labial retraction, persistent protrusion of the tongue and deviation of the tongue away from the injected side.

The abnormal movements were exacerbated when the animals were disturbed and appeared less in repose. The animals appeared to be aware of their limb dyskinesia for they would sometimes hold the affected limb with a normal one. They did not show any signs of distress. The duration of the dyskinesia was potentially a few hours. As a matter of routine, however, once the pattern of dyskinesia had become established, the animals were sedated or anaesthetized. There invariably followed an uneventful recovery with no apparent after-effects.

Location of injection sites associated with chorea. Unequivocal chorea was observed on 62 separate occasions following injections of bicuculline at 54 intracerebral sites (*see* Table and Appendix). The great majority of these sites lay in either the putamen (28 sites) or the lateral segment of the globus pallidus (16 sites). A few positive sites were found immediately below the lentiform complex in the substantia innominata and amygdala, or in the medial segment of the globus pallidus.

Within the lateral segment of the globus pallidus, most injections (70%) gave rise to chorea. Within the putamen, however, chorea was a less common finding (46%). Sites from which chorea could be provoked tended to be located in the medial half of the putamen (fig. D), whereas injections into more laterally placed sites either were without effect or gave rise to myoclonus (*see* below). There was also a tendency for chorea-related sites to be clustered in the ventral half of the putamen (fig. D, F), more dorsal injections often being without effect or inducing myoclonus. Typically, sequential injections through the implanted cannula were made at progressively deeper sites. Using this protocol, injections at the top of a cannula track might be without effect or induce myoclonus, while injections lower down the same track would induce chorea.

The latency to onset of unequivocal dyskinesia varied considerably between injection sites. The site with the shortest latency (1 min, site 79) lay in the lateral segment of the globus pallidus. A review of the regional distribution of sites ($n = 11$) from which chorea could be provoked with a latency of 5 min or less showed that these were all located in the ventral part of the lentiform complex, clustered around the lateral medullary lamina, which separates the putamen from the lateral segment of the globus pallidus. Injections made at sites within either the lateral segment of the globus pallidus or the putamen, and located within 1.5 mm of the lateral medullary lamina, gave dyskinesia with mean latencies of approximately 18 min for either structure.

Somatotopy within the lentiform complex. Dyskinesia could be elicited in the upper limb, the lower limb, or the orofacial musculature, either alone or in any combination. Dyskinesia of the lower limb was produced most readily. Reviewing all the sites which gave chorea, the lower limb was involved at 42 sites, the upper limb at 27 sites and the orofacial muscles at 10 sites. The body part which exhibited dyskinesia with the shortest latency was the lower limb at 30 sites, the upper limb at 16 sites and the orofacial muscles at 9 sites.

There did not appear to be any strict correlation between the regional location of injection sites and the appearance of dyskinesia in a particular body part. Dyskinesia in arm, leg or face might follow injections into either the dorsal or ventral parts of the lentiform complex, and scrutiny of sites which gave dyskinesia in arm, leg or face with the lowest latency did not reveal a consistent pattern. However, when consideration was given only to those sites from which abnormal movements of the arm, leg or face were elicited in isolation, then a clearer indication of somatotopic organization within the lentiform complex became apparent. Although dyskinesia of the lower limb was seen with injections placed throughout the dorsoventral extent of the lentiform complex, dyskinesia of either the upper limb (5 sites) or of the face (5 sites), as the sole dyskinetic phenomenon, was only observed with injections placed in the ventral half of the structure.

Controls. Controls were performed for both the anatomical and pharmacological specificity of injections which induced chorea. As detailed in the previous section, chorea could not be provoked from all injection sites. Chorea was encountered only following injections into the putamen, globus pallidus, or immediately adjacent nuclei. Injections into white matter, such as the corona radiata and extreme capsule, were generally without effect. Furthermore, heterogeneity was observed in the effects of injections made in different parts of the same structure. In the putamen, for example, only injections into the medial and/or ventral parts, in general, gave rise to chorea. Injections placed more laterally and/or dorsally were usually either without effect or induced myoclonus, which is a clearly different form of dyskinesia.

Control injections of vehicle (saline) were made to exclude the possibility that dyskinesia might be produced due to a nonselective effect of volume. This was performed at 2 sites where the dyskinesia was of relatively short latency and either moderate or severe in intensity (sites 27 and 45). At both sites, injection of vehicle was entirely without effect on motor behaviour and at both, saline injections were both preceded and followed by injections of bicuculline. On all occasions bicuculline injection gave rise to prominent and unambiguous chorea.

Myoclonus

Nature of the dyskinesia. Myoclonus was characterized by abrupt, rapid, and brief muscle contractions or jerks, occurring in a repetitive and predictable manner. Involvement of the upper limb was most common (10 of 12 sites), although both upper and lower limbs were simultaneously involved in synchronous myoclonic activity following some injections (3 of 12 sites). The movements always occurred on the side contralateral to the injection. In some instances the involvement of axial muscles in myoclonic activity, either alone (2 of 12 sites), or in association with limb myoclonus (5 of 12 sites), caused spasmodic postural asymmetry and torticollis. In all instances, the movements were initially mild and relatively infrequent (recurring perhaps every 30 s or so). With the passage of time, however, the movements became both more frequent and more severe, in that the force and extent of the movements were increased. As with chorea, once the pattern of dyskinesia became clearly established, the animals were heavily sedated or anaesthetized.

Location of injection sites associated with myoclonus. Virtually all of the sites (9 of 12) from which myoclonus could be evoked lay in the putamen. Additionally, 1 lay in the lateral pallidum and 2 in the corona radiata. The sites which gave myoclonus tended to be concentrated in the lateral half of the putamen. Those which gave myoclonus as the only dyskinetic phenomenon were all in this location.

The physical manifestations of myoclonus and chorea were quite different, as is clear from the descriptions given in the preceding sections. Furthermore, sites from which one or the other could be elicited tended to be clustered in different parts of the lentiform complex. In certain instances, sequentially deeper injections along the same track gave rise first to myoclonus and then to chorea. This phenomenon is illustrated by injection sites 69–73 (fig. C) and 35–37 (fig. F). On a number of occasions, however, both forms of dyskinesia were observed to occur simultaneously following a single injection. This occurred following injection into the putamen at site 74 and into the lateral pallidal segment at site 79.

DISCUSSION

Experimental chorea

The results of this study confirm and extend our previous preliminary reports (Crossman and Jackson, 1984; Crossman *et al.*, 1984; Jackson and Crossman, 1984) that choreiform dyskinesia can be produced in the conscious monkey by injection of GABA antagonists into the lentiform complex. The dyskinesia induced in this manner has predictable and reproducible physical characteristics which closely resemble chorea in man. When present in a mild or moderate form the movements involve the orofacial muscles and/or predominantly the distal parts of the extremities but, when severe, the more proximal limb muscles and the muscles of the limb girdles are recruited so that the dyskinesia becomes virtually indistinguishable from the choreiform dyskinesia and hemiballism which can be induced in the monkey by injection of GABA antagonists into the subthalamic nucleus (Crossman *et al.*, 1980, 1981, 1984). Dyskinesia of the lower limb was seen more frequently, and induced more readily, than dyskinesia of the upper limb. Evidence was found for a somatotropic organization in the lentiform complex, with the head represented most ventrally. This observation agrees with the more detailed somatotopy revealed in careful microstimulation and electrophysiological recording studies (DeLong and Georgopoulos, 1981; Alexander and DeLong, 1985) and in anatomical tracing experiments (Künzle, 1975).

Site of drug action in chorea

The precise site of drug action in the production of chorea in the present study is not entirely clear, solely from analysis of effective injection sites. It is striking that sites from which chorea could be provoked were not homogeneously distributed throughout the lentiform complex, but lay in particular subregions. Effective sites were found throughout the lateral segment of the globus pallidus, but within the putamen they were in general located in the medial part of the nucleus. The best putaminal sites tended to be clustered in the ventromedial area, adjacent to the lateral medullary lamina which separates the putamen from the lateral segment of the globus pallidus. It thus appears, at first sight, as though GABA antagonism in either the lateral pallidal segment or the medial part of the putamen may induce chorea. It may be questioned, however, whether the putamen was the primary site of action following medial putaminal injections. A putaminal site of action would indicate an important functional heterogeneity between different areas within the putamen, since laterally placed injections rarely gave rise to chorea, but rather induced myoclonus.

An alternative hypothesis is that the primary site of action in the production of chorea was the lateral segment of the globus pallidus. It may be of significance that the site with the shortest latency for dyskinesia was located there. Effective injections centred in the putamen could be explained by diffusion of drug through the lateral medullary lamina into the lateral pallidum. This is presumably also the explanation for dyskinesia evoked from sites outside, but close to, the striatum such as the corona radiata, substantia innominata and amygdala.

A few injection sites in the medial segment of the globus pallidus also gave rise to dyskinesia. The number of such sites tested in the present study was, however, low. In a more extensive investigation of the medial pallidal segment, reported previously (Crossman *et al.*, 1984), dyskinesia was not observed. It is, therefore, our overall impression that blockade of GABA transmission in the medial pallidal segment does not consistently induce chorea, and that the instances presently reported are best attributed to diffusion of drug into the adjacent lateral pallidal segment.

Neural mechanisms in chorea

The hypothesis of a primary site of action in the lateral segment of the globus pallidus is an attractive one from a number of points of view. It would be in accord with certain pathological and neurochemical data in Huntington's disease, and with other studies of experimental dyskinesia in the monkey. In Huntington's disease the neostriatum bears the brunt of basal ganglia involvement and there is extensive loss of striatopallidal and striatonigral projection fibres (Bruyn, 1968; Gebbink, 1968). There is considerable evidence, from electrophysiological and neurochemical studies, that the striatopallidal pathway is inhibitory upon pallidal neurons (Yoshida *et al.*, 1972; Ohye *et al.*, 1976) and that it utilizes GABA as its primary transmitter (Fonnum *et al.*, 1978*a*; Nagy *et al.*, 1978), in association with certain peptides. Ligand binding studies on postmortem human brain suggest that Huntington's disease is associated with abnormally reduced release of GABA in the globus pallidus (Penney and Young, 1986). Thus the binding of radiolabelled muscimol to GABA A sites, and of flunitrazepam to GABA receptor-linked benzodiazepine sites, are increased in the pallidum of the Huntington's brain (Penney and Young, 1982; Whitehouse *et al.*, 1985). Pharmacological blockade of striatopallidal GABA transmission in the monkey, by injection of bicuculline into the lateral pallidal segment, might thus reasonably be expected to induce the same physiological effect on lateral pallidal neurons as occurs in man following degeneration of striatal neurons projecting to this structure. As a matter of fact, the same end result could also be envisaged if bicuculline acted in the putamen to block recurrent inhibition and thus cause depolarization blockade of putaminopallidal neurons. This is a realistic possibility, since depolarization blockade is probably the mechanism by which GABA antagonists injected into the subthalamic nucleus prevent firing of subthalamic cells and induce ballism (J. Féger, personal communication). Should either of these mechanism of action prove to be correct, it would indicate that the model of chorea presented in this report is a functionally relevant model for Huntington's disease in man. Such a model has not been available hitherto and would be of considerable potential value for future experimental studies on the pathophysiology of chorea.

The proposed mechanism would effectively cause relative overactivity of lateral pallidal neurons, due to the loss of inhibition from the striatum. Anatomical tracing experiments indicate that by far the most conspicuous efferent projection of the lateral pallidal segment is directed to the subthalamic nucleus (Kim *et al.*, 1976; Carpenter *et al.*, 1981*a*, *b*). The pathway appears to be largely inhibitory upon subthalamic cells, from the evidence of electrophysiological studies (Ohye *et al.*, 1976; Kita *et al.*, 1983), although excitatory responses have also been recorded (Tsubokawa and Sutin, 1972). With regard to the transmitter released by this pathway, the globus pallidus contains high levels of both GABA (Fahn and Côté,

1968) and its synthesizing enzyme glutamic acid decarboxylase (GAD; Oertel and Mugnaini, 1984). Pallidal GAD immunoreactivity is found within both axon terminals (presumably originating from the striatum) and within intrinsic cell bodies. The subthalamic nucleus contains much lower levels of GAD immunoreactivity and this appears to be almost exclusively restricted to terminals rather than cell bodies (Oertel and Mugnaini, 1984). Lesions of the globus pallidus in the cat (the equivalent of the lateral pallidal segment in primates) apparently cause a reduction of GAD in the subthalamic nucleus (Fonnum *et al.*, 1978*b*), although this has been contested in similar experiments in the rat (Van der Kooy *et al.*, 1981). In electrophysiological studies in the rat, the inhibition of subthalamic neurons, induced by stimulation of the globus pallidus, is blocked by ionophoretically-applied GABA antagonists (Rouzaire-Dubois *et al.*, 1980). The available evidence, therefore, points mainly, but not exclusively, to GABA as the transmitter of the inhibitory pallidosubthalamic pathway.

In the mechanism of chorea presently proposed, abnormally increased activity of lateral pallidal neurons would, therefore, lead to increased GABA-mediated inhibition of the subthalamic nucleus. A clear link with well-accepted dyskinetic mechanisms is thus established—it is well known that lesions of the subthalamic nucleus, in both man and monkey, give rise to choreiform and ballistic movements (Whittier and Mettler, 1949).

Relationship between chorea and ballism

It is proposed that experimental chorea, provoked by blockade of GABAergic striatopallidal fibres terminating in the lateral pallidal segment, is mediated through physiologically-induced inhibition of the subthalamic nucleus. This would account for the striking similarity between the physical characteristics of dyskinesia induced by subthalamic and pallidal manipulation.

It is entirely speculative, of course, as to whether similar mechanisms pertain in human chorea. Our proposal that this is the case is intuitively appealing in that it tends to unify our concepts of basal ganglia function. It also addresses a longstanding controversy in clinical neurology concerning the relationship between chorea and ballism. The unity or separation of these dyskinesias has been hotly debated, one perhaps extreme view being that ballism is qualitatively different from chorea and occurs exclusively in association with lesions of the subthalamic nucleus. Most authorities, however, recognize more similarities than differences between the conditions but the lack of objective data, or knowledge of the underlying neural mechanisms, has made it impossible to reach firm conclusions. If the present hypothesis is correct then chorea and ballism are mediated by common neural mechanisms, as we have previously proposed (Mitchell *et al.*, 1985; Crossman, 1987; Crossman *et al.*, 1987), and the conditions are separated only in the quantitative sense. The theory would, nonetheless, allow for considerable differences in the physical expression of dyskinesia provoked by different pathologies. Complete and sudden destruction of the subthalamic nucleus by, for example, discrete vascular accident would be expected to give rise to the most gross and severe form of dyskinesia. Gradual destruction of the enormous, topographically organized striatopallidal projection would be expected to lead, via the lateral pallidal segment, to physiological inhibition of subregions of the subthalamic nucleus. This in turn would manifest itself as dyskinesia less violent than ballism, perhaps restricted to a particular body part. The theory of common neural mechanisms would also account for the otherwise perplexing observations that ballism may sometimes be observed in conditions more commonly associated with chorea (Huntington's disease, levodopa-induced dyskinesia) and that chorea may be exhibited after amelioration of acute hemiballism (Klawans *et al.*, 1976).

Preliminary studies of the regional brain uptake of 2-deoxyglucose (2-DG) tend to support our hypothesis regarding the neural mechanism of experimental chorea. Thus in hemichoreic monkeys, there is increased uptake of 2-DG in the contralateral subthalamic nucleus (Mitchell *et al.*, 1985; Crossman, 1987; Crossman *et al.*, 1987). This is most likely due to increased activity of the terminals of pallidosubthalamic neurons, originating in the lateral pallidal segment at or near the provocative injection site. This view is further supported by the finding that injection of horseradish peroxidase into lateral pallidal sites, from which chorea can be induced, gives rise to anterograde transport of enzyme to terminal fields in the subthalamic nucleus which exactly correspond with the area of increased 2-DG uptake (Crossman *et al.*, 1987).

Subthalamopallidal relations and the nature of the transmitter released by subthalamic neurons

As described above, it would appear that GABA antagonists injected into, or near, the lateral pallidal segment induce choreic movements, whereas similar injections into the medial segment rarely do so. The failure of GABA blockade in the medial pallidum consistently to induce dyskinesia is somewhat surprising in view of the considerable direct and circumstantial evidence which would predict such an outcome. First of all, overwhelming evidence points to the central importance of the medial pallidal segment in the mediation of dyskinesias such as hemiballism and chorea, in both man and monkey (for review, *see* Crossman, 1987). Secondly, some electrophysiological and pharmacological data on the nature of subthalamopallidal relations suggest that the subthalamopallidal transmitter may be GABA. Thus stimulation of the subthalamic nucleus has usually been reported to give rise to inhibition of pallidal neurons (Yoshida *et al.*, 1971; Larsen and Sutin, 1978; Perkins and Stone, 1980; Hammond *et al.*, 1983). Iontophoretic application of GABA antagonists apparently blocks this inhibition (Perkins and Stone, 1981; Rouzaire-Dubois *et al.*, 1983). It would be expected, therefore, that interruption of subthalamopallidal transmission in the medial pallidal segment, by injection of GABA antagonists, would replicate the effect of a subthalamic nucleus lesion, and thus induce dyskinesia.

The fact that this is not generally true gives rise to the question whether GABA is, in fact, the subthalamopallidal transmitter. Indeed, immunocytochemical studies have indicated that, although the subthalamic nucleus contains GAD, it would appear to be localized in axon terminals (presumably derived from the lateral pallidal segment) and absent from subthalamic cell bodies (Oertel and Mugnaini, 1984). It has been suggested, on the basis of 2-DG uptake studies in experimental movement disorders in monkeys, that the subthalamopallidal transmitter is excitatory, rather than inhibitory (Crossman *et al.*, 1987). This proposal arose from two observations. (1) In experiments where dyskinesia was induced by reduction of activity of the subthalamic nucleus (chorea and ballism), there was a concomitant reduction in activity of neurons in the medial pallidal segment, as evidenced by 2-DG uptake in their terminal projection fields (Mitchell *et al.*, 1985; Crossman, 1987; Crossman *et al.*, 1987). (2) In experimental parkinsonism induced by MPTP, where the neurons of the subthalamic nucleus appear to be abnormally overactive, the medial segment of the globus pallidus is, likewise, overactive (Crossman *et al.*, 1985, 1987; Mitchell *et al.*, 1986; Crossman, 1987).

Although information in the literature is rather scant on the subject, there is other evidence that the subthalamopallidal pathway may be excitatory and that its transmitter may be glutamic acid (Kitai and Kita, 1987; *see also* Penney and Young, 1986).

Myoclonus

In addition to chorea, myoclonus could also be induced following injection of bicuculline into the lentiform complex. Effective sites lay within the putamen or immediately adjacent to it. Within the putamen, sites which gave myoclonus generally lay more lateral and dorsal than those giving chorea. The two forms of dyskinesia were quite different and readily distinguishable. They would appear to have separate anatomical substrates within the lentiform complex and, therefore, probably separate underlying mechanisms. This was strikingly demonstrated by certain tracks where injections at successively deeper sites gave rise to a changing pattern of dyskinesia. For example, sites in the group 35–37 and the group 69–72 were injected through single, respective, cannula placements. Within each group, the more dorsally placed injections gave rise to myoclonus alone while the more ventrally placed injections gave chorea alone. Following injections at intermediate sites, both dyskinesias coexisted simultaneously.

Myoclonus can be induced by pharmacological manipulation or pathological abnormality at many levels of the neuraxis. The disorder is by no means exclusive to the basal ganglia. Whether myoclonus induced from basal ganglia sites shares common neural mechanisms with other forms of myoclonus is not known. Myoclonus has been reported previously following injection of GABA antagonists into the striatum of the rat (e.g., Tarsy *et al.*, 1978). In these studies there was uncertainty over the site of action. Apart from the possibility of a primary site of action in the striatum, alternative explanations for the induction of myoclonus were diffusion of drug to the overlying cerebral cortex or a combination of drug action in the striatum and structural damage to the overlying cortex. In the present study, cortical damage was minimal. Diffusion of drug to the cortex seems highly unlikely as the cause of myoclonus because of the considerable distances involved in the monkey compared with the rat. A primary site of action in the striatum, therefore, seems to be the most likely explanation, although involvement of the cortex cannot be excluded.

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APPENDIX

*Characteristics of dyskinesia induced by injection of bicuculline at each of the sites individually identified in fig.**

			Chorea						*Myoclonus*					
			Upper limb		*Lower limb*		*Orofacial*		*Upper limb*		*Lower limb*			
Injection site code	*Structure injected*	*No. of obs.*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Axial/facial latency*	*Notes*
1	GPl	3	++	13/25	++	8/25	—	—	—	—	—	—	—	—
2	GPl	1	—	—	+	27	—	—	—	—	—	—	—	—
23	Put	1	+	27	+	20	—	—	—	—	—	—	—	—
24	Put	1	+	25	++	7	—	—	—	—	—	—	—	—
25	Put	1	++	17	++	6	—	—	—	—	—	—	—	—
26	Put	1	++	8	+	67	—	—	—	—	—	—	—	—
27	Put	3	++/+++	3/7	++/+++	12/40	—	—	—	—	—	—	—	—
28	Put	1	++	4	++	24	—	—	—	—	—	—	—	—
29	Put	1	+	4	—	—	—	—	—	—	—	—	—	—
30	Put	1	++	30	—	—	—	—	—	—	—	—	—	—
31	Put	1	—	—	—	—	—	—	++	9	—	—	14	—
32	Put	1	—	—	—	—	—	—	+	177	—	—	13	—
33	Cor Rad	1	—	—	—	—	—	—	+	10	—	—	9	—
34	Put	1	—	—	—	—	—	—	++	8	++	18	—	—
35	Put	1	—	—	—	—	—	—	nr	32	—	—	—	a
36	Put	1	+	32	—	—	—	—	—	—	—	—	—	—
37	Put	2	+	16	—	—	+	13	—	—	—	—	—	—
38	Put	1	+	35	+	21	+	5	—	—	—	—	—	—
39	Put	1	—	—	+	10	—	—	—	—	—	—	—	—
40	Put	1	—	—	—	—	++	8	—	—	—	—	—	—
41	Put	1	—	—	+	23	+++	10	—	—	—	—	—	—
42	Put	1	+	37	—	—	—	—	—	—	—	—	—	—
43	GPl	1	—	—	+	30	—	—	—	—	—	—	—	—
44	GPl	1	++	17	++	15	+	17	—	—	—	—	—	b

[continued overleaf

APPENDIX (*cont.*)

			Chorea						*Myoclonus*					
			Upper limb		*Lower limb*		*Orofacial*		*Upper limb*		*Lower limb*			
Injection site code	*Structure injected*	*No. of obs.*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Axial/facial latency*	*Notes*
45	Sl	3	+/ ++	8/ 12	+/ ++	11/ 13	—	—	—	—	—	—	—	—
46	Put	1	—	—	+	45	—	—	—	—	—	—	—	—
47	GPl	1	+	53	+++	20	—	—	—	—	—	—	—	—
48	GPl	1	—	—	+	28	—	—	—	—	—	—	—	—
49	Put	1	+	15	+	22	—	—	—	—	—	—	—	—
50	Cor Rad	2	—	—	—	—	—	—	+/ +++	22	+/ +++	22	—	c
51	Put	1	—	—	—	—	—	—	nr	29	—	—	—	d
52	Put	1	—	—	+	50	—	—	—	—	—	—	—	—
53	Put	1	—	—	+	36	—	—	—	—	—	—	—	—
54	GPl	1	—	—	+	18	—	—	—	—	—	—	—	—
55	IC	1	—	—	—	—	—	—	—	—	—	—	—	e
56	GPl	1	—	—	—	—	—	—	—	—	—	—	—	e
57	GPl	1	+	10	+	> 60	—	—	—	—	—	—	—	—
58	GPl	1	—	—	++	12	—	—	—	—	—	—	—	f
59	GPl	1	—	—	—	—	+	25	—	—	—	—	—	g
60	GPm	1	+	37	—	—	+++	7	—	—	—	—	—	h
61	GPm	1	—	—	—	—	+	14	—	—	—	—	—	i
62	Sl	1	—	—	—	—	++	2	—	—	—	—	—	—
63	Sl	1	—	—	—	—	++	2	—	—	—	—	—	b
64	GPl	1	—	—	+	8	—	—	—	—	—	—	—	—
65	GPl	1	—	—	+	25	—	—	—	—	—	—	—	—
66	GPl	1	—	—	+	20	—	—	—	—	—	—	—	—
67	GPl	1	—	—	++	17	—	—	—	—	—	—	—	—
68	Sl	1	—	—	+	20	—	—	—	—	—	—	—	—
69	Put	1	—	—	—	—	—	—	+++	4	+	32	20	j
70	Put	1	—	—	+	36	—	—	—	—	—	—	26	j
71	Put	1	—	—	+	30	—	—	—	—	—	—	25	j
72	Put	1	—	—	++	23	—	—	—	—	—	—	—	—
73	Amyg	1	—	—	+	20	—	—	—	—	—	—	—	—
74	Put	1	+	16	—	—	—	—	+	4	—	—	11	k
75	Put	1	—	—	+++	3	—	—	—	—	—	—	—	—

Injection site code	*Structure injected*	*No. of obs.*	*Chorea*						*Myoclonus*					
			Upper limb		*Lower limb*		*Orofacial*		*Upper limb*		*Lower limb*		*Axial/facial latency*	*Notes*
			Severity	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*	*Severity*	*Latency*		
76	Put	2	++	2/ 14	+/ ++	16/ 36	—	—	—	—	—	—	—	—
77	Put	1	—	—	+	40	—	—	—	—	—	—	—	—
78	Amyg	1	+	2	+	38	—	—	—	—	—	—	—	—
79	GPl	1	++	1	++	1	—	—	+	10	—	—	—	—
80	Put	1	—	—	+	24	—	—	—	—	—	—	—	—
81	Put	1	+	2	+	2	—	—	—	—	—	—	—	—
82	Sl	1	+	3	+	3	—	—	—	—	—	—	—	—
83	Amyg	1	+	40	+++	27	—	—	—	—	—	—	—	—
84	GPl	1	++	15	+	40	—	—	—	—	—	—	—	—

* The severity of dyskinesia was classified as mild and/or occasional (+), moderate and/or intermittent (+ +), severe and/or continuous (+ + +) Latency to onset of unambiguous dyskinesia is given in minutes. Abbreviations as in the legend to the fig, except Amyg = amygdala; Cor Rad = corona radiata

a = spasmodic torticollis to the contralateral side developed after 18 min; b = turning to the contralateral side also observed, c = myoclonic movements of the head to the ipsilateral side also appeared with a latency of 1-2 min, developing into sustained torticollis, d = myoclonic movements of the head to the ipsilateral side also observed, developing into spasmodic torticollis; e = contralateral torticollis and circling behaviour commencing after 25 min; f = leg movements not typical of chorea elicited from other sites; dyskinesia characterised by sharp flexion of the hip and knee followed by extension; g = contralateral torticollis and circling commencing after 15 min, orofacial dyskinesia was limited to contralateral protrusion and elevation of the tongue; h = orofacial dyskinesia consisted of persistent tongue protrusion to the contralateral side, together with constant chewing movements, contralateral circling commenced after 35 min, i = orofacial dyskinesia limited to persistent tongue protrusion to the contralateral side; j = myoclonus of axial musculature causing flexion of head and trunk to the ipsilateral side; k = mild axial myoclonus moving head to ipsilateral side; nr = not recorded.

Brain (1988), **111**, 1235–1245

MYOCLONUS IN A PATIENT WITH SPINAL CORD TRANSECTION

POSSIBLE INVOLVEMENT OF THE SPINAL STEPPING GENERATOR

by B. BUSSEL, A. ROBY-BRAMI, PH. AZOUVI,
A. BIRABEN, A. YAKOVLEFF *and* J. P. HELD

(From the Service de Rééducation Neurologique and INSERM, Unité 215 'Handicaps Moteurs Neurologiques et Croissance', Hôpital Raymond Poincaré, Garches, France)

SUMMARY

A patient with clinically complete cervical spinal cord transection developed rhythmic myoclonic movements of the trunk and lower limbs, demonstrating that, in man, such movements can be generated within the spinal cord itself when deprived of supraspinal control. Electromyographic (EMG) recordings used to define the features of the myoclonus, which had a frequency of 0.3–0.6 Hz, was bilaterally symmetric, and involved extensor muscles. The EMG bursts always appeared in phase in all muscles involved. Peripheral stimulation of flexor reflex afferents (FRA) could induce, slow or interrupt the rhythmic activity. When FRA stimulation induced a flexion reflex, it occurred between extensor EMG bursts and induced alternating flexion-extension activity which could be sustained for several cycles. Soleus and quadriceps monosynaptic reflexes were depressed during the silent period of the rhythmic activity. Several arguments, mainly the great sensitivity of the myoclonus to flexor reflex afferent stimulation, suggest that the myoclonus observed in this patient was due to partial release of a spinal stepping generator.

INTRODUCTION

Spinal myoclonus in man has been reported repeatedly since its first description by Lhermitte (1919). As reviewed by Halliday (1967), it is characterized by repeated involuntary spontaneous contractions with a rhythmic or semirhythmic character linked to a primary abnormality within the spinal cord itself. In contrast to other types of myoclonus reviewed by Halliday (1967) (*see also* Halliday, 1975; Marsden *et al.*, 1982), little is known about its pathophysiology and treatment (but *see* Frenken *et al.*, 1976; Garcin *et al.*, 1968; Henry *et al.*, 1973; Silfverskiöld, 1986). We have investigated a patient with a clinically complete spinal cord transection who developed myoclonus, which was unusual in that it was widely distributed to muscles innervated by spinal segments below the level of transection. Such an exceptional observation shows that, even when deprived of supraspinal influence,

Correspondence to Professor B. Bussel, Clinique de Rééducation Motrice, Hôpital Raymond Poincaré, 92380 Garches, France.

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the human spinal cord can generate rhythmic activity on its own. This study reports the electrophysiological investigations performed in this patient which suggest that the rhythmic activity is generated by the release of a physiological spinal rhythm generator.

CASE HISTORY

Ch. F., a 23-yr-old man, sustained severe head and spinal cord injuries in a traffic accident in September 1983. On admission he was deeply comatose due to the head injury. A CT scan performed on the same day showed multiple diffuse and bilateral hyperdensities and cerebral oedema. The patient was recorded as having a tetraplegia below C7 level, and cervical x-rays showed a vertebral fracture at C7. He remained comatose for 9 days. Ten months after the trauma, he had a single epileptic seizure which did not recur; he was given 100 mg of phenobarbital daily. A 4-yr follow-up revealed a slight persistent memory disorder as the only cerebral dysfunction. He has had no cerebellar or oculomotor disorder, or palatal myoclonus. When performed long after the trauma, the EEG and a cranial CT scan were within normal units.

The neurological deficit due to the spinal cord injury did not regress during the long-term follow-up. Neurological examination performed 6 months after the accident showed that the patient had a clinically complete spinal cord transection at C7-C8 segmental levels. The motor deficit was partial at the C7 level, and complete below, where no voluntary movement was observed. There was hypoaesthesia below C8 and complete anaesthesia below T3. A peripheral radicular deficit was observed at C7-C8-T1 levels: the left triceps tendon jerk was absent and the first dorsal interosseous muscles were atrophied on both sides. The number of spinal cord segments damaged seemed to be limited to C7, C8 and T1, since he did not have Horner's syndrome and noxious stimulation below the lesion induced bilateral sweating and vasodilatation that spread bilaterally to the face, indicating the integrity of the T2-T4 segments. Below C8-T1 level, spinal reflexes were present and exaggerated on both sides: the tendon jerks in the lower limbs were brisk and percussion of the crest of the pubis induced a contraction of the rectus abdominis. Toe twisting induced both withdrawal reflexes of the lower limbs and a contraction of the abdominal wall; sacral reflexes were exaggerated.

Magnetic resonance imaging (fig. 1) showed an apparently complete section of the cervical spinal cord at the C6-C7 vertebral level in accordance with the clinical findings. Below this level the spinal cord appeared to be normal.

RESULTS

Description of the rhythmic movements

In January 1985, 15 months after the spinal cord injury, the patient developed rhythmic extension movements of the trunk and the lower limbs. These movements appeared at a time when the patient spent most of the day lying in bed because of major hypotensive episodes. The strength and the frequency of these rhythmic contractions, and the number of muscles involved, varied from day to day. Sometimes, rhythmic extension of trunk, hip, knee, ankle and toes appeared spontaneously and lasted for hours with a constant frequency of 0.6 Hz. Rhythmic activity was observed in upper erector spinae but never in muscles innervated by spinal roots above C7. The contractions were strong enough to induce abnormal movements of such force as to propel him from his bed or his wheelchair. During these contractions, the trunk was always displaced backwards without any lateral

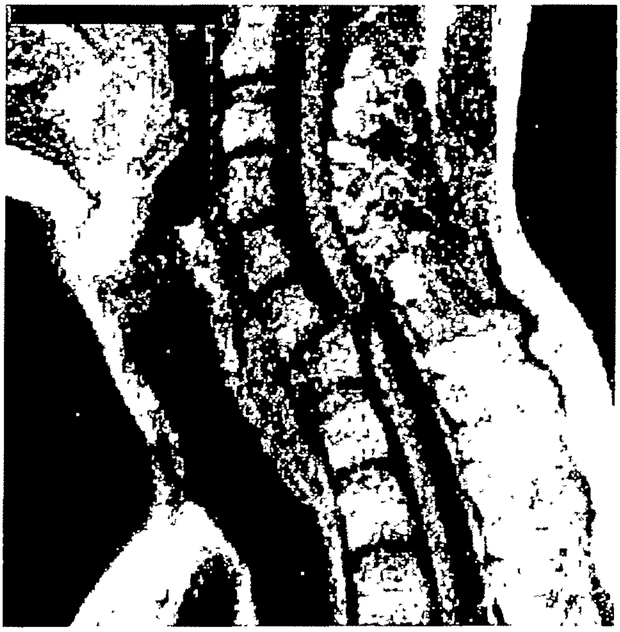

FIG. 1. Magnetic resonance imaging of the spinal cord lesion (sagittal spin echo image, TE = 28 ms, TR = 300 ms) showing fracture of C7 vertebral body with posterior displacement, a focal weak signal within the spinal cord at the same level, and normal appearances of the spinal cord below the lesion

curvature, indicating symmetry of muscular contraction on the two sides. On other days, the rhythmic activity only appeared after stimulation (mainly shaking of the whole body), had a slower and more variable frequency (0.3–0.5 Hz) and would vanish after a few minutes. Sometimes the rhythmic contractions could be detected only by palpation of the posterior trunk muscles. No rhythmic movement was observed during sleep.

Electromyographic studies

The pattern of the rhythmic activity was investigated electromyographically over several recording sessions between February and July 1985. Electromyographic (EMG) recordings were obtained by using surface adhesive electrodes placed on the degreased skin over the muscles. The signals were recorded on a magnetic tape recorder and then analysed by means of an analogic-digital converter linked to an Apple II microcomputer. Direct paper recordings were also used.

Rhythmic EMG activity (fig. 2) was observed in the trunk muscles (longissimus thoracis) and in the posterior lower limb muscles (gluteus maximus, soleus, hamstrings) but not in the antagonistic muscles (rectus abdominis, quadriceps, tibialis anterior). This shows that the rhythmic activity was characterized by a wide distribution in posterior extensor muscles. Some involved muscles were pure extensors (longissimus thoracis, gluteus maximus, soleus), others such as the

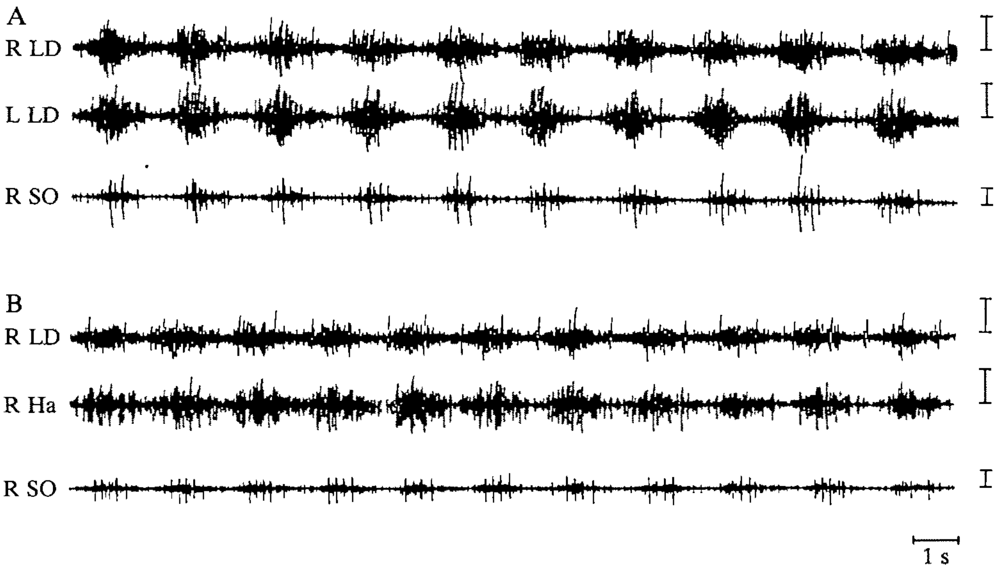

FIG. 2 Examples of rhythmic EMG activity observed spontaneously A, symmetric EMG bursts. B, extension of rhythmic activity in extensors. The muscles recorded are indicated on the left. R = right, L = left, LD = longissimus thoracis, SO = soleus, Ha = hamstrings The time base is indicated at the bottom of the figure. Calibration bars (1 mV) are shown on the right.

hamstrings were partial extensors (hip extensors as well as knee flexors). Frequently tonic activity was recorded between the rhythmic EMG bursts. The rhythmic EMG activity was not dependent on ventilation since it was observed during apnoea; moreover, it was verified that its frequency did not depend on the respiration rate (recorded by means of a strain gauge) or on the heart rate.

The onset times of the EMG bursts and their duration were only measured for longissimus thoracis, where the minimal interburst tonic activity permitted automatic analysis. The EMG activity was digitalized (sample frequency 100 Hz) and rectified. Calculations were performed on 1 min recordings which corresponded to 35 EMG bursts. It appeared that the duration of the EMG burst was inversely correlated with the duration of the *following* silent period ($r = 0.78$) but not with the duration of the *previous* silent period ($r = 0.12$). The duration of each period (that of one burst and its following silent period) had a mean of 1700 ± 85 ms, which suggests that each cycle begins at the onset of the EMG burst.

The visual inspection of the EMG recordings showed that the EMG bursts appeared to be nearly synchronous in all the muscles involved, irrespective of their side or of their distance from the spinal cord (fig. 2A, B). The relative onset times of the bursts in the different muscles were not measured. However, we observed on long duration recordings (more than 3 min) that the phase shift between the rhythms in the different muscles was stationary, which indicates that the frequency of the rhythmic activity was the same in all muscles.

Effects of peripheral stimulation

Stimulation above the level of spinal transection did not modify the myoclonus, provided it did not cause trunk movement. Conversely, the rhythmic activity was greatly sensitive to peripheral stimulation applied below the level of the transection.

Some stimuli could induce the appearance of the rhythmic activity. Trunk movements induced by brisk shaking of the whole body or deep voluntary inspiration were probably responsible for the repetition of rhythmic movement episodes in the patient's daily life. Stimulation of the distal limbs, such as by twisting

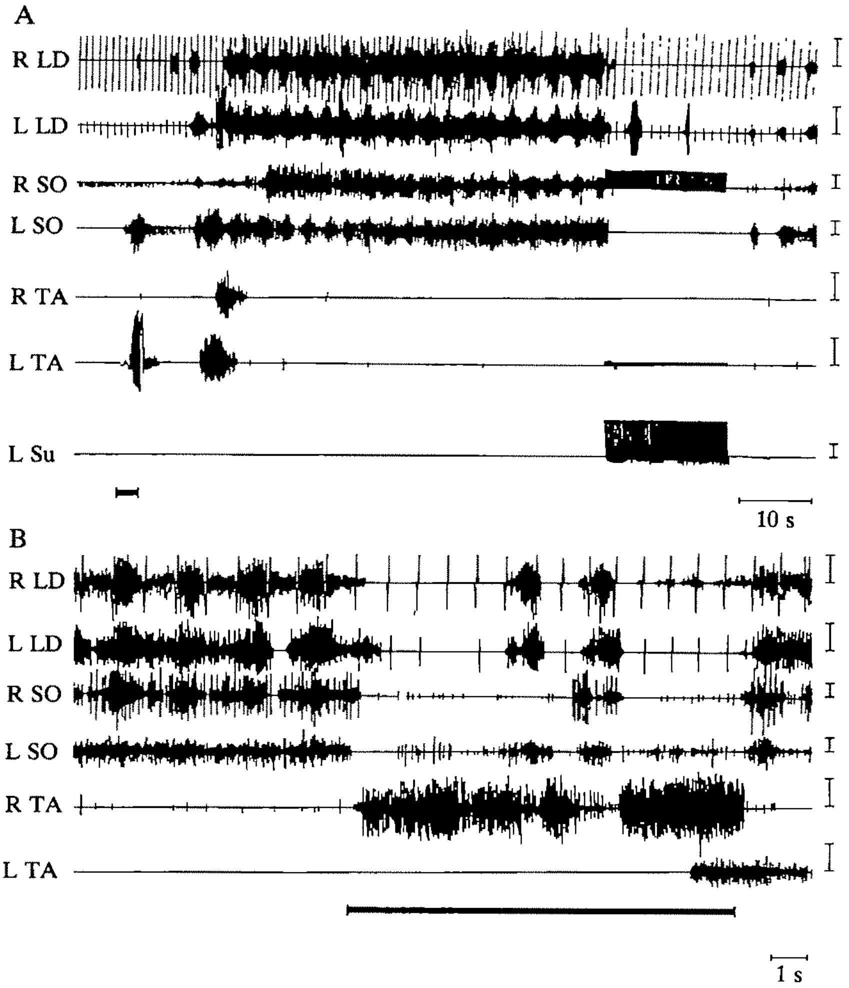

FIG. 3. Effects of peripheral stimulation on the rhythmic EMG activity. A, *left side* mechanical stimulation of the left foot (toe twisting) applied at rest. *Right side* electrical stimulation applied to the left sural nerve (1500 ms train, 1 ms pulses at 200 Hz, 25 mA). B, mechanical stimulation of the right foot (toe twisting). The horizontal bars indicate the duration of the mechanical stimulation. The muscles recorded are indicated to the left TA = tibialis anterior, L Su = output of the current probe measuring the intensity of the left sural nerve stimulation. Other abbreviations as in fig. 2 Calibration bar = 10 mA for the current probe output.

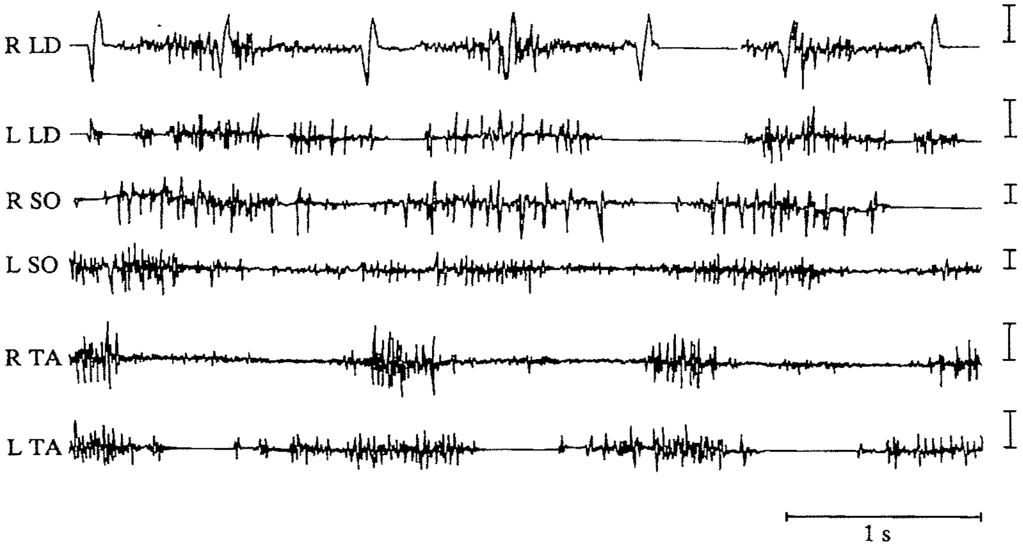

FIG 4. Rhythmic alternating EMG activity. EMG observed 10 s after mechanical stimulation (not illustrated) of the left foot (toe twisting). Abbreviations and calibration bars as in fig. 3.

the toes, could also induce the appearance of rhythmic movements and an ipsilateral flexion reflex (fig. 3A). Unilateral hip flexion could also induce the myoclonus or increase both its amplitude and its frequency. However, similar stimulation could also induce prolonged suppression of the rhythmic activity. Both electrical (fig. 3A) or mechanical (fig. 3B) stimulation of flexor reflex afferents (FRA) were effective, irrespective of the occurrence of a flexion reflex. When it reappeared, the rhythmic muscle activity had a much lower amplitude and a slower frequency. Bilateral hip flexion also abolished the myoclonus.

Low intensity stimulation acted by modulating the frequency of the rhythm in all muscles discharging rhythmically. A sustained soleus stretch obtained by manual plantar pressure induced a decrease in the frequency of the longissimus thoracis rhythmic EMG activity which was 0.6 Hz before and 0.5 Hz during the stimulation. Similar results were obtained with cutaneous light tactile stimulation or with thermal stimulation (such as rubbing an ice cube) applied on the back or on the lower limbs. In all cases, slowing of the rhythm of the myoclonus was observed in all muscles involved.

The temporal relationship between the flexion reflex discharge and the myoclonus is of particular interest. If a flexor response was obtained after FRA stimulation, it appeared between two extensor muscle EMG bursts (fig. 3B). Similarly timed activity was frequently observed for several cycles as a poststimulus after-discharge and constituted a true alternating flexor-extensor pattern of activity (fig. 4) which was never observed to occur spontaneously.

Electrical stimulation of the FRA regularly interrupted the EMG discharge in

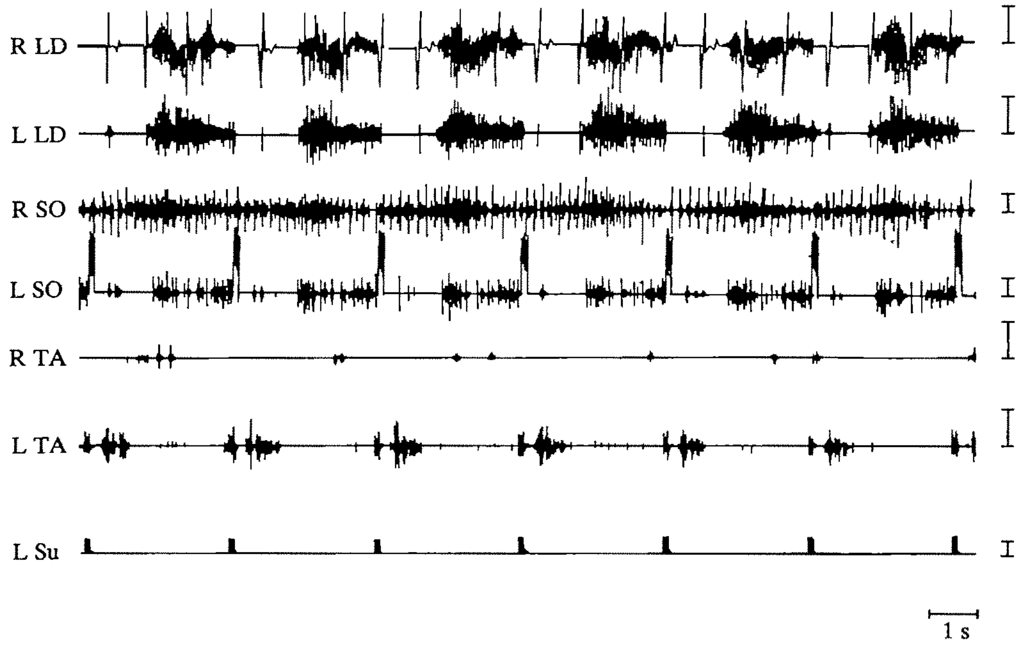

FIG. 5. Effects of repetitive electrical stimulation of the FRA. Electrical stimulation (100 ms trains, 1 ms pulses at 200 Hz, 10 mA) applied to the left sural nerve. Note the stimulus artefact on the left soleus recording. Abbreviations and calibration bars as in fig. 3.

extensors 70 ms later. When applying repetitive FRA stimulation (at 0.3 Hz, fig. 5), each sural nerve stimulus evoked a late flexion reflex in the ipsilateral tibialis anterior (latency 200 ms, duration 600 ms) and simultaneously a silent period of about 1 s in both longissimus thoracis and the ipsilateral soleus. Repetitive stimulation with frequencies of 0.5 and 0.25 Hz, which were lower than the spontaneous frequency of the EMG activity (0.58 Hz), could drive the rhythmic activity. In contrast, stimulation at a higher frequency (1 Hz) caused a total inhibition of EMG activity.

Peripheral stimulation usually induced bilateral and nearly symmetric modifications of the rhythmic activity. However, after FRA stimulation, alternating right-left activity could occasionally be observed for one cycle. More frequently, unilateral tonic EMG responses were observed, mainly after unilateral hip flexion or toe twisting.

Monosynaptic reflex studies showed that the amplitude of the soleus H reflex was increased during EMG bursts and decreased during the following silent period. This also applied to the quadriceps H reflex, although it was not involved in the rhythmic activity.

Treatment

When the rhythmic movements appeared, the patient was treated with phenobarbital (100 mg/day) and diazepam (10 mg/day). The dosage of diazepam was increased to 20 mg/day for an 8 day period without benefit. A single dose of clonazepam (1 mg subcutaneously) had no additional effect. Clonidine was also used since it was reported to reduce automatic spinal motor activity in patients with a spinal cord section (Tuckman *et al.*, 1982). In our patient, a single subcutaneous injection of clonidine (0.075 mg) had no noticeable effect. Treatment with L-DOPA and with a decarboxylase inhibitor (750 mg/day, orally) was maintained for 3 weeks without clinical effect. In contrast, carbamazepine had a dramatic effect: the rhythmic activity disappeared totally after 3 days of treatment (600 mg/day, orally). This effect lasted throughout the 15 days of treatment; the dose was then progressively decreased, the movements reappearing 2 days after its complete withdrawal. Sodium valproate (1.5 g/day, orally) had the same effect: the rhythmic activity disappeared after 3 days of treatment. It was continued for 2 months with the same efficacy. The drug was then progressively withdrawn without any recurrence of the rhythmic activity. Now, after a follow-up of 2.5 years, the rhythmic movements have still not reappeared. However, the patient still complains of occasional extensor spasms, without any rhythmic features. The clinical state of the spinal reflexes remains unchanged.

DISCUSSION

In the clinical case reported here, clinical and MRI data strongly indicate that the spinal cord transection was complete. Spinal myoclonus appearing below a spinal transection is exceptional. Lhermitte (1919) described spinal myoclonus with a similar frequency (0.53 Hz) in a patient with a complete, anatomically verified, spinal cord transection at T7-T9 levels. The myoclonus, which involved mainly the lower abdominal muscles (T10-T12), could also spread to the lower limb muscles (*see also* Tuckman *et al.*, 1982). Other cases of spinal myoclonus have been described as being segmental and have been reported in patients with incomplete spinal lesions (Halliday, 1967; Garcin *et al.*, 1968; Henry *et al.*, 1973). Diffuse myoclonus has been related to diffuse spinal lesions whatever their cause (Frenken *et al.*, 1976; Silfverskiöld, 1986).

Since the myoclonus appeared below the level of a spinal transection it can be accepted that the rhythm is fully generated at spinal level, although the possibility that the generation of the rhythm is due to a focal spinal lesion below the level of the transection cannot be excluded. Spinal myoclonus resembling that observed in our patient has been described in the cat after a localized lesion due to the intraspinal inoculation of Newcastle virus (Luttrell and Bang, 1958). In our case, as in the experimental model (Luttrell *et al.*, 1959), several mechanisms could underlie the rhythmic activity. The role of peripheral reafference, producing repetitive reflex activity, cannot be completely eliminated, but appears unlikely. Since clonus is known to have a much higher frequency, the role of Ia reafference volleys may be demoted. The FRA have access to various reflex pathways (Lundberg, 1979) which act on contralateral or ipsilateral extensor muscles (Holmqvist, 1961). Since these pathways have a classical reciprocal organization it appears unlikely that they induce a symmetric activation of extensor muscles. Some other propriospinal pathways supplied by FRA (Jankowska *et al.*, 1973) could activate extensor muscles in a bilateral manner. Their role in the generation of the rhythm again cannot be excluded. However, it would seem paradoxical if FRA stimulation, which regularly induces flexion reflexes, was responsible for a sustained rhythmic extension activity. The most likely mechanism to explain the generation of the rhythm is that of a central rhythm generator acting on the different extensor motoneuron pools.

In cats and dogs, it has been shown that the lumbar spinal cord isolated both from supraspinal centres and peripheral influences, can generate rhythmic movements such as scratching or locomotion (Brown, 1911-12, 1912-13; *see* review in Grillner, 1975). The rhythmic movements observed in our patient are different from those observed in normal human locomotion. However, spinal stepping generators may induce distinct rhythmic activities as well as the typical alternating pattern of normal locomotion (Grillner and Zangger, 1979; Koehler *et al.*, 1984; Orsal *et al.*, 1986). Some features observed in these rhythmic patterns were also found in our patient. Spinal locomotion and the myoclonus observed in our patient both greatly depend on peripheral stimulation. Spinal locomotion is usually triggered by FRA stimuli (Forssberg and Grillner, 1973; Viala *et al.*, 1974; Grillner and Zangger, 1979) and may be inhibited by peripheral stimuli (Viala and Buser, 1971); its frequency can be modulated by peripheral stimulation of these afferents (Forssberg and Grillner, 1973; Grillner and Zangger, 1979). In our patient we observed a late flexion reflex (Roby-Brami and Bussel, 1987), similar to that observed in the cat (Andén *et al.*, 1964) which in turn is related to spinal locomotion (Grillner, 1973; Viala *et al.*, 1974; Lundberg, 1981).

In conclusion, this observation shows that the human spinal cord deprived of supraspinal influences may generate rhythmic activity, probably through the activation of a central pattern generator. In view of the regularity of the rhythm and of the sensitivity of the activity to FRA stimulation, it is assumed that this generator could be the spinal stepping generator.

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We are grateful to Ch. F. who accepted numerous recording sessions despite the discomfort of his condition. We also thank Professor G. Frija, from the Raymond Poincaré Hospital, who performed and interpreted the magnetic resonance imaging and B. Guezard for his technical assistance. This work was supported by grants from INSERM.

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BOOK REVIEWS

Functional Recovery in Neurological Diseases. *Advances in Neurology*, Volume 47. Edited by S. G. Waxman. 1987. Pp 639. New York. Raven Press. Price $118.00.

The neurologist of popular myth resembles a war correspondent with a slightly unhealthy interest in uncontrollable catastrophies. This reputation is perpetuated in Britain by the fact that neurologists have little time to do anything other than write down what has gone wrong. There is not much space in the clinic for the improving patient, despite the fact that many of the most important neurological conditions such as stroke, multiple sclerosis and head injury are characterized by substantial (but largely mysterious) functional recovery.

Concepts such as neural plasticity and functional adaptations have often been used as slogans by enthusiasts of neurological and cognitive rehabilitation, but the biological evidence has been thin compared with the large clinical effects it is supposed to explain. By contrast, this book is scientifically purposeful, and by no means a polemic. As a whole it bears out the editor's premise that recently 'clinical studies and basic science had begun to converge so that one could plan a single volume integrating the two perspectives'.

Among many valuable contributions are those reviewing clinical topics such as peripheral nervous system regeneration, and those describing basic work in areas such as the biophysics of injured and demyelinated axons, remyelination, axonal sprouting and synaptic plasticity. There is also useful material at a physiological-functional level, for example on recruitment of ineffective synapses after injury, and on intrinsic spinal cord locomotion—with neuropharmacological data. Other neuropharmacological topics would not have been out of place. For example, the adaptive mechanisms following unilateral nigrostriatal lesions would seem to have potentially more general relevance. As the book shows at many points, consideration of functional recovery often involves two other great neurobiological themes (which are equally under-represented in the neurological academic tradition): development and degeneration. The effect of age on potential for functional recovery is a topic which, although frequently alluded to, might have been reviewed specifically Differences between children and adults are sometimes striking. Those between younger and older adults are less so, judging by data on recovery from dysphasia. However, there are interesting potential interactions with ageing processes, such as the possibility that late deterioration following polio might be due to a late failure to sustain an adaptively enlarged neuronal territory.

The selection of therapeutic topics is slightly quirky, but stimulating. The review of neural implantation encourages optimism in the context of the book as a whole. Cognitive rehabilitation is represented by a final paper on 'cognitive orthoses': presumably the term should apply not only to computer programs but also to good books like this one.

Christopher D. Ward

Wolff's Headache and Other Head Pain. Fifth edition. Edited by Donald J. Dalessio. 1987. Pp. 434. New York and Oxford: Oxford University Press. Price £50 00.

Headache: Problems in Diagnosis and Management. *Major Problems in Neurology*, Volume 15. Edited by Anthony Hopkins. 1988. Pp. 398. London: W. B. Saunders. Price £30.00.

The classical work of Wolff, first published in 1948, set a unique and distinctive example of the specialized monograph on headache It was not only an exhaustive exposition of the whole subject as it was understood at the time, but it conveyed the individual flavour of the author's extensive and original experimental work. The succeeding 1963 edition followed this pattern and both were universally and justly acclaimed. Thus in preparing his own updated versions, Donald J. Dalessio has bravely confronted an enormous challenge.

This fifth edition is revised and includes new chapters on the genetics, pathophysiology and epidemiology of migraine, and chronic paroxysmal hemicrania (Sjaastad). Advances in imaging and radiology are covered by Zyroff and Seat. The 25 chapters range widely and embrace allergy, diseases of the eyes, nose, teeth and jaws; neurosurgical aspects are covered by chapters on brain tumour, raised intracranial pressure and the surgical treatment of pain in the head and neck. The neurologist is served by accounts of head pains arching from the cervical spine, toxic, vascular and arteritic causes as well as psychiatric chapters on stress, personality and behavioural therapy. To achieve this coverage, Dalessio has secured the aid of experts, many from the USA, but also others from three continents. There is the inevitable unevenness of writing. The scientific content varies from the critical analytical approach of J. W. Lance to the impressionism of Seymour Diamond. The latter's appraisal of muscle contraction headache leans heavily on depression and a variety of nonspecific endocrine tests which many would regard as nondiagnostic, expensive and time consuming; treatment is based on the empirical use of MAOI and tricyclic drugs. Ziegler adopts a similar style. He acknowledges the importance of adequately controlled drug trials but provides insufficient reference to them in discussing various remedies, and, surprisingly, he still recommends ergotamine by mouth and in doses repeated half-hourly up to 5 mg/attack.

This remains a valuable and very nicely produced book, remarkable for its comprehensive approach—in the spirit of H. G. Wolff. But it is a smaller book than the 1963 edition, and this contraction in size in the face of expansion in knowledge is in some ways a paradox and results in a work with too little basic biochemistry to assist an understanding of the mechanisms and too little pharmacology to explain the necessarily empirical therapy. The young trainee will search in vain for detailed clinical descriptions, for example of hemiplegic and ophthalmoplegic migraine. These aspects could be usefully expanded and the softer science of respondent, reflexive and operant behaviour could be sacrificed without inflicting grievous loss to the reader.

Anthony Hopkins' book addresses a similarly broad view of headache as opposed to a study of migraine. It follows in the wake of other British authors who have traversed the same seas, most recently Blau's comprehensive and larger tome.

This work is almost identical in length to Dalessio's and covers the same syndromes, though there is less on local disorders of the eyes, nose and cranial neuralgias. Epidemiology is well described by Hopkins and by Ziegler (the only author appearing in both books). There is a similar weighting of attention to psychosocial factors (Feinemann, Fitzpatrick and the editor). The main difference in this area is that the British work is much more critical and objective, pointing out the limitations of these methods and explicitly exposing the shortcomings of doctors in their attempts at explanations and reassurance to patients.

Classification, a shibboleth for the migrainologist, remains problematical: the division between tension headache and common migraine is difficult, but the rejection of classic migraine as an entity, merely the most severe end of a continuous spectrum, is fraught with dangers and ignores the observation of regional spreading oligaemia which is confined to classic migraine. The pathophysiology is well covered by Lauritzen and Nicholson, though as in the American book it would have been helpful to have an account of the protean biochemical accompaniments and triggers of migraine, even if their aetiological primacy is in doubt. Platelets may have justifiably lost their recent popularity as the main focus of biochemical disorder but brief mention is surely justified. Similarly, Serotonin receives the briefest mention in the psychiatric section as a factor in reducing central pain, and prostaglandins do not figure in the index. The surgical treatment of pain in the face by Jannetta deals almost exclusively with trigeminal neuralgia and with microvascular decompression. The editorial note which follows—in almost apologetic vain—attempts to rectify the lack of balance. Giant cell arteritis is superbly reviewed by Ross Russell and Graham. Kelly provides a scholarly if not wholly unbiased view of plaintiffs and their headaches after cranial trauma, based on his vast experience of this contentious problem.

These comments highlight a healthy state of progress and disagreement in the headache world, a state impossible to envisage twenty years ago when the waters were more muddied but stagnant. Both are excellent books and the neurologist will covet both for his bookshelves. If one had to choose, Hopkins, if shorter on descriptive material, has the advantage of a new book with a fresher approach, and at £30 it is better value

J. M. S. Pearce

Infections of the Nervous System. Edited by P G. E. Kennedy and R. T. Johnson. 1987. Pp. 284. London: Butterworths. Price £32.50

This is Volume 8 of the Butterworths International Medical Reviews. As the editors of this multiauthor book explain in their introduction, the last few years have seen the discovery of new infections of the CNS and the recognition of a broader spectrum of disease caused by organisms with which we are already familiar, expanding their pathogenicity as a result of host immunosuppression. There have also been diagnostic advances, in imaging and in immunology, and new therapeutic agents have been discovered. Information on each of these is available in original articles and reviews in relevant journals, not always easily accessible to the neurologist; so it is timely for a comprehensive account of CNS infections to appear in one volume.

Thirteen chapters have been written by 17 authors from the UK, USA, Thailand and Mexico. Those on bacterial infections include meningitis, CNS tuberculosis, spirochaetal diseases, botulism and tetanus. Viral meningitis, encephalitis and their complications, slow virus infections, AIDS and immunosuppression are covered in separate chapters. Fungal disease is dealt with comprehensively in an excellent chapter, and parasitic disease is selectively represented by cerebral malaria and neurocysticercosis.

Inevitably, in a multiauthor publication, the quality of the chapters varies: none is bad. All include adequate clinical descriptions, relevant contemporary developments, and comprehensive and appropriate bibliography. In general the illustrations are clear, well produced, and illuminate the text, except for the algorithm of neurocysticercosis, which I felt allowed the surgeon too much scope for vacillation!

I particularly enjoyed reading three chapters. 'Cerebral malaria' is written convincingly by people with practical experience and dispels some of the pathophysiological myths which have surrounded the syndrome. The chapter on spirochaete disease contains a brief and useful review of syphilis, a comprehensive account of Lyme disease, and descriptions of the relapsing fevers and leptospirosis. The neurological complications of varicella-zoster virus are elucidated in an extremely comprehensive and masterly review

Each chapter of this book stands on its own, and there is no overlap or needless repetition. The editors are to be complimented for this and for producing a fresh and topical volume which will not quickly be out of date. It will appeal particularly to neurologists, specialists in infections and tropical diseases, and other physicians, and to laboratory staff. There is much information and good sense for medical students.

It is reasonably priced, and I warmly recommend it.

Milne Anderson

Focal Peripheral Neuropathies. By John D. Stewart. 1987. Pp. 434. New York: Elsevier. Price $55.00.

There seems to be no end to the continuous spate of neurological monographs that emerge from the medical book publishing houses. Some are good, some are indifferent, some are awful. This is one of the better ones. A monograph, to justify its appearance, has to provide something additional to what can be gleaned from the longer neurological textbooks or handbooks, apart from the convenience of having the topic available between the covers of a single book. A more comprehensive treatment of the subject than is possible in a textbook is one advantage and this is certainly provided within its terms of reference by *Focal Peripheral Neuropathies*. Accordingly, it is not the slim volume type of monograph, running, as it does, to 434 pages. Nevertheless, it is agreeably priced at $55.00. It is primarily written from the standpoint of a medical neurologist. Acute nerve injuries which require surgical management; and peripheral nerve tumours, therefore, do not receive extensive coverage.

The book begins with three short chapters covering the basic anatomy and pathological processes, both of injury and recovery, relevant to focal neuropathies. This is followed by a chapter on the evaluation of the patient with a focal neuropathy, including the use of nerve conduction studies. The main body of the book consists of a systematic treatment of focal nerve lesions, beginning with those of the spinal roots and limb girdles and proceeding out to the periphery, first for the upper limbs and then the lower, and lastly dealing with focal neuropathies affecting the trunk. Each chapter, prefaced by a short section on the relevant anatomy, considers the clinical features, diagnosis and management. The book ends with chapters on multifocal neuropathies and neuropathic pain, and an Appendix on the dermatomal and peripheral nerve cutaneous distributions and the myotomal and peripheral nerve innervation of commonly tested muscles.

This is a useful addition to the growing number of monographs on neuromuscular disorders. The illustrations are informative and the book is clearly if somewhat simplistically written. It is copiously referenced.

P. K. THOMAS

Inflammatory Disease of Muscle. Edited by Frank L. Mastaglia. 1988. Pp. 203. Oxford: Blackwell. Price £39.50.

The inflammatory myopathies comprise an interesting group of disorders that, although heterogeneous, are sufficiently well circumscribed to merit monographic treatment. This emerges from the useful opening chapter in which Sir John Walton provides a general overview and classification, and an appraisal of the problems encountered in making a differential diagnosis between these disorders, and between them and noninflammatory myopathies. As anyone working within the field of neuromuscular disorders is aware, this is not always an easy task. The second chapter on muscle biopsy by Roger Pamphlett outlines the indications for biopsy, the choice of muscle and the techniques that are currently available. It is curious in that the biopsy specimens with which he has chosen to illustrate the chapter do not include a single example of an inflammatory myopathy. This is followed by a justifiably substantial chapter by Ira Targoff and Morris Reichlin on immunological aspects.

The bulk of cases encountered by clinicians consist of examples of polymyositis or dermatomyositis, either alone or in conjunction with a variety of connective tissue disorders and neoplasia. These are comprehensively covered in chapters by Harvey Sarnat, and Neil Rosenberg and Steven Ringel, on juvenile dermatomyositis and adult polymyositis and dermatomyositis, respectively, a separation justified by clinical differences. Forms of myopathy other than polymyositis occurring in connective tissue disorders are dealt with in a further chapter by Paul Zilko.

Direct viral and bacterial infections of muscle are uncommon, as are parasitic infestations of muscle in developed countries. They are none the less important. These interesting conditions are covered in well-presented chapters by Samuel Chou, Victor Ojeda and Frank Mastaglia, and David Grove. The book is completed by a chapter on miscellaneous conditions such as sarcoidosis that fall outside the coverage of the preceding chapters.

Despite some inevitable overlap between chapters, admitted by the editor, *Inflammatory Diseases of Muscle* provides a cohesive, authoritative and informative account of this interesting group of disorders. It is beautifully produced and remarkably free of typographical errors—although I liked 'distil' for 'distal'. I would have preferred 'Bornholm disease' for 'Bornholm's disease', considering the derivation of the name.

P. K. THOMAS

Clinical Neuroendocrinology—Edition 2. By Joseph B. Martin and Seymour Reichlin. Contemporary Neurology Series. 1987. Pp. 759. Philadelphia: F. A Davis. Price not stated.

This is the second edition of a proper textbook of neuroendocrinology and not the proceedings of a symposium in disguise. It is written entirely by the two main authors of the first edition published ten years ago at a time when neuroendocrinology had barely found a clinical identity. Excellent though it was, the first edition was devoted almost exclusively to pituitary disease with a good deal more endocrine than neurological input. This edition goes a long way towards correcting the imbalance, chiefly by the addition of chapters devoted to neuroregulatory peptides and to psychiatric aspects of neuroendocrinology. Whilst many chapters remain unchanged, others have been extensively revised, particularly those dealing with posterior pituitary disorders and neuro-ophthalmology.

In common with the first edition, the format and the whole approach to the subject reflects the interest of both authors in basic neuroscience and their belief that a proper understanding of disease depends upon the interaction between science and clinical medicine. The first hundred pages of the book are devoted to the anatomy, pharmacology and neurophysiology of the hypothalamic-pituitary unit. The pituitary is then considered in detail in a series of chapters headed 'Regulation of Secretion' with respect to TSH, ACTH, prolactin and growth hormone, again with a considerable physiological bias. The strength of this approach is self evident in neuroendocrinology because of the clear link between the laboratory and advances in clinical management. An obvious example is the use of bromocriptine in the treatment of prolactinomas which can be seen as a direct consequence of the discovery that dopamine is the physiological prolactin inhibiting factor. There is, however, a disadvantage in this approach which may be more evident to neurologists than to endocrinologists, and that is the relative paucity of clinical information. There is, for example, no description of the clinical manifestations of Cushing's disease or of acromegaly, let alone the neuromuscular complications seen by neurologists. Thus the section on growth hormone secretion does not refer to entrapment neuropathy, generalized neuropathy or to spinal cord disease secondary to skeletal changes. Likewise, the neuromuscular complications of thyrotoxicosis, the cerebellar syndrome of hypothyroidism and myxoedema madness are not to be found. Indeed neuropathy, which might be regarded as an important presentation of neuroendocrine disease, is not listed in the index. In the same vein the absence of a chapter on the neuroendocrine complications of diabetes is perhaps the most glaring omission from the point of view of the clinician.

The emphasis on neuroscience is also apparent in the new section on neuroregulatory peptides. The distribution of a variety of peptides is considered in detail but within the 50 pages or so there is only a brief description of the neurochemistry of Parkinson's disease and Huntington's chorea. The final chapters on psychoneuroendocrinology and the endocrine response to stress make rather arid reading. Neuroimmunology is briefly considered but without mention of the commonplace such as myasthenia gravis or the nonmetastatic complications of carcinoma

In spite of the criticism that this book is too 'academic' in its approach, it is probably the best textbook of neuroendocrinology currently available. The references in particular are exhaustive and it can be recommended to neurologists as an authoritative key to the literature when confronted by neuroendocrine disease.

N. F. LAWTON

Disorders of the Pituitary. By G. T. Tindall and D. L. Barrow. 1986. Pp. 498. St. Louis, MO: C. V Mosby and Oxford: Blackwell. Price £68.00.

The pituitary gland has become an area of difficulty for many neurosurgeons. For a long while it seemed that the neurosurgical management of pituitary disease had largely stabilized: the endocrinology, pathology and modes of treatment appear to be well established. However, in the last 10 years or so all has been thrown into confusion. The pathological and endocrinological bases of pituitary disease have been subject to a basic reappraisal and have proved to be very much more complex than the older neurosurgeons assumed. The re-emergence of the extracranial surgical approaches to the pituitary fossa has meant that the neurosurgeon has had to acquire new and somewhat problematical technical skills, and the therapeutic approaches to the various types of pituitary tumour have altered without the development of any clear consensus, notably in the case of prolactin-secreting tumours.

The time could hardly be more opportune for the appearance of a clearly written review of what is currently known about the pituitary gland based on the point of view of the neurosurgeon. This book fills the requirements magnificently. Written by two eminent American neurosurgeons, it covers every aspect of the pituitary. Initial chapters deal with the normal anatomy and physiology of the gland and these are followed by clear accounts of the pathology and radiology of pituitary disease and the methods of endocrine assessment available. Those diseases of the gland which are of interest to the neurosurgeon are thoroughly reviewed in turn and the final chapters deal in detail with the surgical and radiotherapeutic techniques and the question of the so-called 'empty sella' syndrome.

Diseases of the Pituitary is beautifully produced and illustrated. The type face is large, easy on the eye and sensibly laid out. The text provides exactly what is needed: a clear, well written and balanced account of what is currently known. Each chapter is followed by a thorough and up-to-date list of references. This reviewer is glad to say that he can find no significant faults in this book. He is glad to possess his own copy and to recommend it to every neurosurgeon. It is not cheap but the purchaser will find that it is money very well spent.

R. S. MAURICE-WILLIAMS

Clinical Neurosurgery, Volume 34. Edited by John R. Little. 1988. Pp. 717. Baltimore and London: Williams and Williams. Price £63.00.

This is the 34th volume of *Clinical Neurosurgery*, based on papers presented at the Congress of Neurological Surgeons at New Orleans in 1986. On that occasion the honoured guest was that remarkable pioneer of microneurosurgery, Professor Gazi Yasargil. As is customary, the honoured guest has contributed opening chapters on 'Reflections of a neurosurgeon', 'Neurosurgical horizons' and 'The surgical approaches to inaccessible brain tumours'. This reviewer approached these chapters with pleasurable anticipation but found them sadly disappointing. The tradition of devoting the opening chapters of *Clinical Neurosurgery* to the musings of eminent neurosurgeons on philosophical or cultural matters is perhaps unfortunate. Such chapters often make painful, indeed sometimes embarrassing reading, bringing home to one the good sense in the old saying that 'the cobbler should stick to his last'

The opening sections apart, this volume provides the usual excellent value of this series. The wide range of topics covered include carotid endarterectomy, a critical reappraisal of the intracranial-extracranial by-pass trial, evoked potentials in neurosurgery, spinal instability, the neurosurgery of AIDS and a number of chapters on tumours of the orbit, the skull base and cerebellopontine angle and the third and fourth ventricles.

There are several chapters devoted to controversial topics in cranial and spinal trauma and also chapters on ultrasonography, and the use of magnetic resonance imaging for the spine. Unfortunately a general lessening in the clarity of neurosurgical writing has meant that some of the authors have done less than justice to the intrinsic interest of their subjects. Welcome exceptions to this general failing are the chapters on spinal arteriovenous malformations and on anterior approaches to the upper cervical spine.

These criticisms apart, however, clinical neurosurgery maintains its usual value for the practising neurosurgeon. As on previous occasions, this reviewer is glad to recommend *Clinical Neurosurgery* as a straight-forward means of keeping up to date with a wide range of advances.

R. S. MAURICE-WILLIAMS

Surgery of the Third Ventricle. Edited by Michael L. J. Apuzzo. 1987. Pp. 872. Baltimore: Williams and Wilkins. Price £149.00.

Over 60 authors have contributed to this extensive volume. The editors aimed to provide neurosurgeons, neurologists and neuroscientists with a single reference which would aid 'comprehension and management of lesions affecting the third ventricular chamber'. The subject is taken in its broadest sense, encompassing not only lesions arising from third ventricular structures but also those which encroach on or distort the third ventricle, including lesions of the skull base, basal cisterns or blood vessels.

The book begins with a comprehensive historical review and is followed with a section incorporating chapters on basic anatomy, embryology, physiology, pathology and radiology of the region. Dr Rhoton's chapter, illustrated and written in the style of his many publications, describes the microsurgical anatomy of the ventricular system, blood vessels, cisterns and skull base in the finest detail. The value of this is self evident. The preceding chapter on comparative anatomy, however, depicting the third ventricular system in the shark, sheep and iguana, can be of little interest to clinicians. In the radiology chapter scans and x-rays are clearly reproduced but it is disappointing that more examples of magnetic resonance imaging of various pathologies are not included. They appear infrequently throughout other chapters.

The bulk of the book concentrates on surgical approaches, their complications and effects on adjacent brain Every conceivable route to the third ventricle is described in detail—through the lateral ventricle, through the corpus callosum, through the lamina terminalis, through the sphenoid, along the sphenoid wing and posteriorly, above and below the tentorium cerebelli. Information abounds but unfortunately repetition frequently occurs. Even in the 'anatomical' chapters on the 'deep veins' and 'microsurgical anatomy', the authors cannot resist describing the various operative approaches. This repetition, however, is not without value; subtle nuances exist between different authors and most authors state their individual preference and their reason for employing a particular strategy. Any defect in the text is readily compensated by the clarity of the numerous illustrations, particularly those relating to the operative approaches Most have been drawn by the same illustrator and their simplicity permits immediate anatomical comprehension

Some chapters focus on specific problems such as the management of craniopharyngioma or of vein of Galen aneurysms. Others review the neurological, psychometric and endocrinological deficits which may arise from surgery in the third ventricular region. Several valuable chapters describe the new developments in CT-guided stereotaxis for both biopsy and for tumour removal. Surprisingly the text includes a chapter on CSF diversion, describing various shunt operations in detail. This seems out of place; any neurosurgeon reading direct approaches to the third ventricle should be well beyond the need for information on shunt techniques!

I believe the editor has achieved his aim He has collected a wealth of information on the management of lesions in and around the third ventricle and produced a reference text of undoubted value to the neurosurgeon although perhaps of more limited interest to the neurologist or the neuroscientist.

K. W. LINDSAY

The Early Story of Alzheimer's Disease. Edited by Katherine Bick, Luigi Amaducci and Giancarlo Pepeu. 1987. Pp. 147. New York Raven Press. Price $66.00.

This fascinating and highly instructive book is the product of collaboration between the Italian Study Group on Brain Aging and the World Federation of Neurology Research Group on the Dementias, both young organizations, the former already responsible for the generation of much research activity in Italy and further afield, and the latter encouraging world-wide collaboration of research in dementia. The book brings together, in highly readable English and with well-produced illustrations, six immensely important publications over a four-year period early in this century which established the essential features of the cortical pathology of Alzheimer's disease. The first is Alois Alzheimer's original brief report in 1906 (published in 1907) to a meeting of the South West German Psychiatrists in Tübingen of a patient with a 'peculiar, little known disease process' in which he found the 'miliary' foci now known as senile plaques and the characteristic neurofibrillary changes which have since carried his name. The choice of the other three authors whose papers have been included might be questioned; Blocq and Marinesco originally described the senile plaque in 1892, and that account would have been of great interest as might that of Redlich (1898).

Oskar Fischer's (1907) detailed, accurate and beautifully illustrated article is chosen and it has to be admitted that his description of the light microscopic appearances of the senile plaque were not substantially added to until the availability of immunocytochemistry. Fischer did not suggest the central homogeneous material of a plaque was of 'amyloid-type', but he did recognize it was a 'peculiar type of deposit... not plasma, nor of neuronal origin, it does not contain calcium or iron . . is neither fibrin nor bacterial', and that it was surrounded by nerve fibres showing nodular, club-like or bulbous proliferative change

Detailed descriptions and illustrations of the neurofibrillary alterations (tangles) of Alzheimer's disease appeared in an article of Francesco Bonfiglio (1908) on a case of cerebral syphilis, the brain being passed on by Alzheimer. Numerous senile plaques were present, as was vascular and microglial proliferation which resembled syphilitic lesions. Bonfiglio wisely withheld judgement, though recognizing the close resemblance to Alzheimer's case.

Emil Kraepelin's (1910) long account of the senile and presenile dementias is largely clinical and extremely complicated, with accounts of the many, if not all the symptoms of mental disturbance which may occur in old age, intermingled with attempts at interpretation in a way which, hopefully, would not be found acceptable as a method of presentation today. Nevertheless, the lengthy description of the mental changes in normal old age and their close similarity with the more severe manifestations of many early cases of senile dementia, the part played by arteriosclerotic disease in producing dementia in old age, and their distinguishing characteristics, clinically, and the effects of small vessel disease on the cortex is clearly described, and many other sections of the account are clear and perceptive.

The two final chapters are by Gaetano Perusini on Histology and Clinical Findings of some Psychiatric Diseases of Older People (1910) and The Nosographic Value of some Characteristic Histopathological Findings in Senility (1911).

In the first paper, at Alzheimer's suggestion, Perusini describes four cases of presenile dementia with similar clinical and pathological features. All had, in common, a severe dementing process and plaques and tangles in the cortex, but abnormalities of the pia and blood vessels were also present, in one case thought to be of syphilitic origin, and engendering considerable discussion of that possibility in the others. Every cellular element of the brain is minutely described in these cases, again in a way unlikely to obtain publication now. Plaques and tangles, and their significance are discussed at length, and a good many conclusions would stand today, particularly that the histological differences between these cases of presenile disease and the commonest form of senile dementia is quantitative only, though cortical neuron loss is confined to the presenile cases. Again the illustrations are comparable with many in contemporary publications.

In the final contribution, Perusini (1911) concentrates particularly on the technological details of tangle demonstration and the methods and importance, with various fixatives and stains, of distinguishing glial reaction from some neurofibril alterations, a complication hardly thought worthwhile mentioning by other authors, though he did emphasize that difficulty mainly arose when tangled remnants survived as skeletons of neurons. Of greater interest is his review of the cases published within a four year period of Alzheimer's original description and the possibility that a newly described and specific disease has been identified. He reacts with scientific caution to that suggestion.

I was delighted by this book. It presents to any English-speaking reader six contributions central to the explosion of knowledge of the psychiatry and neuropathology of old age which occurred almost 80 years ago and which have previously been available (and not together, to my knowledge) only to fluent readers of German and Italian. The editors, the translator Katherine Bick, and her collaborators, and the individuals and organizations which conceived and financed this publication have my profound admiration, gratitude and congratulations.

B. E. TOMLINSON

Neurolinguistics and Linguistic Aphasiology. By David Caplan. 1987. Pp. 498. Cambridge: Cambridge University Press. Price £30.00; paperback £10.95.

In the introduction Caplan takes trouble to explain the title of the book, which at a first glance appears to be a duplication. What he calls neurolinguistics is more commonly called clinical aphasiology Neurolinguistics is an unfortunate hybrid of a term abused, among others, by some cranks who sell 'improved communication'. Other uses of 'neurolinguistics' also have little to do with neurology, but Caplan indeed makes a credible go at merging neurology with linguistics.

The first chapter deals with philosophical issues such as reductionism. The computer analogy is a timely one to use, even though comparing the brain to hardware and mental operations to software is probably still very far off the target. The chapter putting Broca's work into perspective is excellent. Caplan's analysis of Wernicke's and Lichtheim's paper is also interesting. The discussion of the revival of connectionism is well done, except it is irritating to see Flechsig's name consistently misspelled in the discussion of Geschwind's papers. The description of the connectionistic model is followed by the hierarchical models, mainly Jackson's and Brown's, and the global models, mainly Pierre Marie's and Goldstein's. A chapter on process models is devoted to Luria.

The section of the book called linguistic aphasiology begins with the linguistic descriptions of aphasic syndromes and a critique of modern taxonomies. In the chapter on lexical semantics, the lengthy review of complex papers will be interesting to many, including some novices willing to read the originals. Apart from an occasional *lapsus linguae*, such as the word 'preservation' misprinted as 'presentation', the chapter provides insight into some recent work including the cascade model of object recognition.

The chapter on phonology deals with phonemes as abstract representations of sound segments which are relevant to the distinction between words in language. Some principles of segmental phonology and how the syllabic and phonological rules interact with the process of word formation are discussed. The abstract linguistic representations of sounds are shown to be relevant for the process of planning and producing words. This chapter also reviews the evidence that malapropisms and semantic errors are similar in their sound pattern to their target in normals and this supports the theories of phonological organization of the mental lexicon. Evidence concerning a system linking phonological and lexical representations is discussed

The alphabetic, consonantal and syllabic orthographies, which are compatible with phonologically mediated reading, are contrasted with ideographic script in the chapter on acquired dyslexia. The renaming of semantic errors and the inability to read nonwords as deep dyslexia created a great deal of subsequent research by virtue of focusing on the alternative routes of reading. Surface dyslexia, which is thought to be a disturbance of the whole word-reading route, is characterized by regularization errors or alterations in the surface structure of words. However, Caplan reviews several alternative explanations in addition to the original theories of deep and surface dyslexia. The evidence that reading can proceed by whole word-recognition route without semantic representation or understanding is incorporated into an overall model of reading.

The much debated topic of agrammatism is viewed in the context of sentence production. The difficulty in distinguishing functional words and content words appears to be still a major theoretical issue. Errors in normal speech (spoonerisms) are the basis of Garrett's popular model of sentence production. This model resembles, to a surprising extent, Arnold Pick's concept of language production from the beginning of this century, and I was wondering why Caplan did not draw the parallel.

The concept of agrammatism in aphasia is complicated by the variability of this phenomenon. Paragrammatism, which is a substitution of grammatical elements rather than their omission, is arguably also part of this syndrome. It is almost as if the linguists are now discovering what clinicians have known for a long time that agrammatism is far from being pure or even being a syndrome. This is a good example of the difficulty with variation of observed phenomena that derives from unstandardized testing. Disturbances of sentence comprehension are described in the context of syntactic structures in great detail, obviously reflecting the author's interest. The computational analogue of the comprehension of sentences is 'parsing' Parsing is a device in computers and it became an aspect of psychological theory of how people understand sentences. Not only a central agrammatism but also an impairment of auditory verbal short-term memory may underlie the failure of comprehending syntax. However, evidence is shown that syntactic comprehension deficit is not secondary to short-term memory loss and to illustrate that specific syntactic comprehension deficits exist, Caplan concludes the chapter with a detailed presentation of his own work using an object manipulation paradigm.

The chapter on cerebral dominance and hemisphere specialization for language is concise, mentioning some highlights selectively. Caplan argues that evidence indicates that the right hemisphere is a computational area for language processing when the requirements exceed the capacity of the perisylvian association area of the left hemisphere. This, however, is based on debatable evidence as to what constitutes a right hemisphere language deficit.

The review of localization discusses the issue of subcortical lesions with aphasia, among others. Some of the localization evidence induces Caplan to state that some core language processing does not take place in the perisylvian association cortex in some individuals. The discussion of the exceptions to regular localization is somewhat incomplete. Papers that emphasize exceptions do not always ensure that the examination on which the classification is based is standardized or that the patients are examined at a certain time from onset. Many of the exceptions are related to recovery or misclassification

Caplan makes the point repeatedly that the classical aphasic syndromes are not homogeneous. However, he underplays the even greater lack of homogeneity among the large number of single cases examined through information processing models. Interestingly, some of the presented evidence indicates that looking at individual symptoms is no more productive than the symptom complexes of classical syndromes. He turns his own negative results around, however, and interprets the widespread lesion location for the syntactic comprehension deficits as indicative of individual variations in the localization of this function, and continues to insist on narrow localization that varies from patient to patient. He dismisses the most probable explanation that functions that will be disturbed by varied lesion location are likely to be distributed in a diffuse network, and it is only the specific aggregation of these symptoms, such as one finds in syndromes, that leads to consistent cerebral lesion localization.

In a summary chapter, Caplan admits that linguistic aphasiology can be carried out with little or no reference to the neurological basis for cognition or language, or the neuropathological determinants of functional abnormalities. He also argues why linguistic aphasiology need not employ group studies. The analysis is much more detailed; therefore it is more practical to examine single cases only. Furthermore, the search is on for dissociations and not for similarities. He does not, however, discuss the possibility that the diversity of approach and the accumulation of dissociations will lead to looking at variability rather than a commonality and an answer to the underlying questions.

Experiments borrowed from cognitive psychology, such as lexical decision tasks and priming with reaction time responses, have become the fashionable way to explore brain function. Extensive experimentation on one or two patients takes the place of the systematic survey of patient population with comprehensive test batteries.

Abstract mathematical models of neuronal interconnection (some displayed in a 'confusional matrix') are presented as models of performance. These models pertain to some extent to the principles of inferring functional localization from lesion data, even though the conclusions are tentatively negative. The interest in interactive activation models involving both parallel and sequential processing has led to more 'boxology' and diagram making. This seems inevitable, even though it must cause Henry Head to rotate in his grave frequently. The overview of the book is an upbeat expression of hope that influences from the basic neurosciences, such as the neurophysiology of elementary psychological functions and computational theories, such as parallel distributed processing models will further advance our knowledge and integrate with the psycholinguistic analysis of normal and abnormal language.

This volume is a highly sophisticated interpretation of a great deal of data. Rather than a text for the novice, it is more an erudite discourse for the expert. It does not deal with certain major issues in aphasiology, such as the severely affected aphasic, residual communication and recovery, substitution and dynamic reorganization and treatment issues. The emphasis is on normal function, how brain modelling can be inferred from deficit phenomena, and clinical issues are only touched upon. The book discusses some aphasic symptoms in detail in relationship to normal processes rather than presenting a general survey of a large number of studies.

Caplan promotes the view that aphasiology should be a derivative of linguistics and psycholinguistics, the exclusivity of which the majority of clinicians and expert aphasiologists may not subscribe to. However, this view reflects the large number of linguists presently in the field. The principles behind linguistic structures have been well worked out in some instances as Caplan states, but they are not an end in themselves to characterize aphasic symptoms. A combination of linguistic, psychological and biological factors of language is needed to understand both clinical and normal phenomena.

This book is a tour de force, not only by its size and the amount of information collected, but its integrative approach. The style is lucid and readable most of the time and only occasionally lapses into lengthy and tangential arguments. It should be read by everyone interested in the field.

ANDREW KERTESZ

Organic Psychiatry. The Psychological Consequences of Cerebral Disorder. Second edition. By W. A. Lishman. 1987. Pp. 745. Oxford: Blackwell. Price £45.00 (cloth), £25.00 (paper).

It is a great pleasure to review the long-awaited second edition of this book. In the Preface Professor Lishman tells us that he has added some 1000 new references to his already extensive bibliography, thus updating what was already a valuable reference book which had no rivals. The publication of the original, in 1978, was a landmark in organic psychiatry, and the book was surreptitiously referred to as one of the best textbooks of neurology around.

The book is divided into two sections; the first relating to principles of examination and investigation of patients, the second to specific disorders. The majority of the new inclusions are to be found in the second part, although in the first an account of, for example, positron emission tomography, magnetic resonance imaging, and other up-to-date investigative techniques are to be found. Illustrations, especially emphasizing the clinical value of some of these for neuropsychiatry, would be welcomed and would help break up the continuous text.

In the second section, the major addition has been to give movement disorders a chapter on their own, recognizing the growing interest in movement disorders generally, and their importance for organic psychiatry in particular.

While some may find it a criticism, others may say that refreshingly absent from the text are major concerns over classification of illness, for example DSM III is mentioned on only one page. Classification of seizures is sparsely mentioned, and the more recent complicated classification of the epilepsies gives way to older versions. Perhaps this is because throughout this remains an essentially clinical book, wide-ranging in the knowledge it provides and being well referenced. It is a book essential for the library of any practising psychiatrist or neurologist, and will continue to be a major reference book for postgraduate courses.

The edition I was sent for review is the paperback one measuring nearly 11 in. in height. This fits uncomfortably, if at all, on the majority of shelves in my library, and certainly I cannot place it next door to the first edition. The publishers should have thought more carefully about their readers when they chose this format, and also must answer for keeping this edition from us for so long. The preface of the second edition is signed June 1985, the book taking over 20 months to reach this reviewer's desk. I would urge Professor Lishman soon to start work on his third edition, and also to seek another publisher.

Michael R. Trimble

The Neuropsychology of Anxiety—An Enquiry into the Functions of the Septo-hippocampal System. By Jeffrey A. Gray. 1982, paperback 1987. Pp. 548. Oxford: Oxford University Press. Price £19.50.

The important theoretical contribution on the *Neuropsychology of Anxiety* published in 1982 by Jeffrey Gray is now available as a paperback. Fortunately, the author has 'resisted the temptation to add new material piecemeal', the argument presented in the book is too complex for that. A preface dated November 1985 updates some points. In my opinion, this book deserves to be read and studied in detail as it is, even today.

Jeffrey Gray has formulated a complete theory of anxiety based on an extensive review of data from animals and humans, both from the physiological and psychological aspects. It is the first comprehensive theory on anxiety and depression that is worked out in detail and is compatible with most of our knowledge in neurobiology as well as in contemporary psychology. The bulk of the behavioural data is from learning experiments in animals, but the theory does rely on clinical findings as well as sophisticated mathematical analyses of personality.

The theory is based on the concept of inhibition, a theme which has been central in behavioural brain research for the last 30–40 years. The term is rooted both in pavlovian theory and in neurological thinking, but did not reach respectability in Western science before Fulton's pupil Kaada mapped inhibitory structures in limbic areas, and later demonstrated the behavioural importance of the structures. The Kaada-McCleary model of response suppression has been the leading model in limbic research since the early 1960s.

Most research in this area concentrated on the response inhibition as it was evidenced in learning experiments. The frontal lobes, the septal area and hippocampus were necessary for learning and memory; lesions produced perserveration. Gray stuck to his own model, postulating that the inhibition was to be understood as the expression of the type of fear that inhibits motor movement, the most extreme being 'freezing' in animals. He suggested, therefore, that these areas of the brain were the target zones for anxiolytic drugs, regardless of class and type. This behavioural inhibition system compares all incoming stimuli with expected events, and expected results of acts. When expectations are not fulfilled, this system interrupts ongoing motor programs. This happens when the organism is subjected to novelty, nonreward, punishment and innate fear stimuli.

So far, Gray's theory does not really go much beyond the mainstream, except for the emphasis on the relation to anxiety. However, in his book of 1982 he managed to encompass knowledge from all aspects of neurobiology. He discussed the anatomical relationships between the septal area, hippocampus, other limbic structures, and the brainstem. Particular emphasis was made on the specific monoaminergic systems ascending from the brainstem, and he discussed the relations between his theory and the biochemical substrate of helplessness and depression. He showed how these neurobiological observations were compatible with knowledge from personality and psychiatric treatment, biochemical as well as behavioural.

Jeffrey Gray's work was extensively reviewed and discussed in *Brain* and *Behavioral Science* in 1982. This is not the place to reiterate any of those arguments. Gray has made a substantial and impressive contribution to our field and it was a pleasure and very profitable for me to read it 6 years later. The book deserves the extended readership made possible by the paperback edition. No serious student of brain and behaviour should be without one.

Holger Ursin

Perception and Communication. By D. E. Broadbent. 1987. Pp 338. Oxford University Press. Price £15.00.

When *Perception and Communication* was first published in 1958, it laid the foundation stones of a whole new view of psychological functioning, that of information processing. Inspired by Broadbent, *Experimental Psychology* ever so gently prised the baton from the fingers of the already ailing *Behaviourism*, and was off and running before the latter was disembowelled by Chomsky's theoretical scalpel. The Broadbentian tradition then crystallized into *Cognitive Psychology* with the publication of Neisser's seminal book in 1966 but, on reflection, this marked the zenith of experimental psychology. It now has very little influence on its most successful of progeny, *Cognitive Science*, which owes a greater allegiance to theoretical analyses of the nature of mind than it does to the kind of empirical psychology advocated in the final chapter of *Perception and Communication*. So, should this reprinted issue be seen as some kind of memorial to a tradition gunned down by the same theoretical forces that put paid to its behaviourist predecessor all those years ago? I sincerely hope not. Re-reading *Perception and Communication* reminded me of why I wanted to do psychology, rather than linguistics, philosophy, or computer science, in the first place. The excitement of experimentation and empirical discovery comes across as a breath of fresh air, nay a trade wind, in what can sometimes appear to be a Sargasso sea of endless computer printouts and interminable philosophical hectoring about our epistemological naivety. The explosion of experimental ingenuity that arrived with information processing psychology raised the possibility that a new window had opened on the mind and all that psychologists had to do was look in to see what was there. It may have been naïve, but it must have been fun.

In Chapter 1, Broadbent skilfully lulls the behaviourists into a false sense of security by claiming that his advocacy of the new communication theory in psychology is no real threat, just another way of looking at the same data. However, by the end of the chapter he has already firmly entrenched radical new concepts such as *capacity*, and shifted the focus away from behaviour to perception. It may not have been disembowelment, but death by poisoning is just as sure. Chapter 2, on selective attention to speech, is, in many ways, the best in the book. The content is still an excellent primer for the thirty years of work that followed. Broadbent's own experiments are classic examples of how to do experimental psychology with simplicity and ingenuity. The synthesis of these experiments with other data demonstrates a grasp of the material unsurpassed by his contemporaries. The main conclusions have also stood the test of time: that limitations on attending are due to central rather than peripheral sensory factors and that the effects vary with information load (albeit that our concept of information load has changed). In Chapter 3 the gloves come off with a full blown exposition on the value of communication theory, introducing new terms such as *information, channel, input, redundancy,* as well as the introduction of prototypical information processing psychology, with its accent on the importance of the processes intervening between the input and the output of the system. It is in this chapter that Broadbent presents the specific theoretical contribution made by the book: the idea that there is a filter at the entrance of the central nervous system which allows some stimuli to pass, while preventing the myriad of other possible stimuli that impinge on the organism from consuming the limited processing resources of the individual.

Much of the rest of the book deals with the operating characteristics of the filter and the implications of the new information processing view both for theoretical and applied psychology. Chapter 4 outlines the data on the conditions under which signals interfere with each other, Chapter 5 explores the effects of noise on perception and Chapter 6 applies filter theory to vigilance tasks. Chapter 7 attempts to explain the differences found between groups such as hysterics, dysthymics and psychopaths, and the effects of traits like extraversion and neuroticism, in terms of the general filter theory, with some speculation on the physiological correlates of these individual differences. Indeed, the major legacy of the kind of psychology advocated in this book is the application of information processing psychology to individual differences, with a great deal of interesting current work on the cognitive nature of depression, anxiety, ageing and intelligence.

Chapters 8, 9 and 10 return to theoretical issues. In Chapters 8 and 10 filter theory is pitched against behaviourist accounts of extinction and reinforcement (and can therefore be ignored by all except historians of psychology). In Chapter 9 Broadbent brilliantly develops his new ideas on attention and memory, arguing, in effect, for an early version of a working memory system. Chapter 11 looks at skill and argues that the human operator is best regarded as a single channel processor of limited capacity. I doubt there would be many cognitive psychologists who would still want to defend the idea of a single channel, and it certainly is at odds with notions like distributed processing and modularity, but, as Professor Posner points out in his foreword, the notion of limited capacity at the highest levels of processing is still very much with us.

Of course, in a reprint of a classic book the chapter that holds the most interest must be the last one 'retrospect and prospect'. Of the 12 principles summarized in this chapter only two, both relating to behaviourist notions and admitted by the author to be the 'most tentative', seem truly archaic. Not bad after thirty years. Of course, many would take issue with the detail of the others but their central concerns, limited capacity, attention, attention switching, memory storage and individual differences are still *the* topics of experimental psychology.

Unfortunately, as I suggested above, experimental psychology is no longer the pack leader in the application of psychology to brain sciences. Part of the reason for the premature demise of the influence of experimental psychology on the emerging cognitive sciences may have been Broadbent's ambivalence towards theory. While it may be true that 'the great value of practical problems is that they force upon us difficulties which experiments done for theoretical reasons may ignore' (basically by curbing 'speculation'), applied questions also quickly lead to pedantry and triviality. The problem is that the only applied questions which have an interesting theoretical content are the *early* questions. Certainly questions about the workplace that have had a theoretical impact on psychology are contained, in toto, in this book. The irony is that Broadbent's searing attack on the value of testing highly specific quantitative predictions from theoretical models could now be directed, thirty years on, at the very movement he founded. An interest in human performance in practical settings, and a fascination with experimental manipulation for its own sake, turned cognitive psychology into the psychology of 'tasks' rather than minds. There is a paradox here. For all Broadbent's insistence on the only good problem being an applied problem, the major contribution of *Perception and Communication* is a theoretical one. It really did change our conception of how the brain might work. Indeed Broadbent has never been slow to take issue with alternative theoretical positions. I suspect that it is not really the applied question that matters as long as the theoretical one has a significant empirical content. Thus, in criticizing what was seen as the immense contribution of Hull, Broadbent points out that his use of Euclidean geometry as a model for science is unfortunate because 'geometry is . . . a logical exercise without any necessary correspondence with reality. It may be useful practice in rigorous thinking... but it is not science'. For Broadbent, then, empirical endeavour is necessary for establishing psychological reality. But science is special because it requires both theoretical and empirical research. Broadbent was undoubtedly wrong in 1958 to suppose that empirical endeavour alone could solve major problems in psychology. For example, I feel sure he would agree (and it is regrettable that this reissue does not contain a reassessment of the core ideas in *Perception and Communication* by Broadbent himself) that his views on language understanding were simplistic and would have benefited from the purely theoretical developments that have taken place in linguistics since then. Cognitive science has generated an explosion of theories as to how minds could conceivably work, and this progress could not have been made without a major contribution from predominantly unempirical sources: philosophy, linguistics and the computer sciences. However, in order to discover how our brains actually work we would do well to rekindle some of Broadbent's passion for empirical work, since scientific progress also requires matching the possible with the actual. The reissue of *Perception and Communication* is a timely reminder of psychology's experimental roots.

MICHAEL ANDERSON

Cerebral Cortex. Volume 6. **Further Aspects of Cortical Function, Including Hippocampus.** Edited by E. G Jones and A. Peters. 1987. Pp. 464. New York· Plenum Press. Price US $69.50.

This series is fast becoming a classic source on the structure and function of the cerebral cortex. From the outset I should make it clear that this is not, of course, primarily a clinical work, although in the present volume, Alzheimer's disease and epilepsies do get some mention. Instead it is a collection of well-written, comprehensive review chapters on the basic issues presented by this most challenging of brain regions

The present volume differs from its predecessors in looking beyond the neocortex to the phylogenically older, and apparently simpler, cortex of the hippocampus. The final two chapters cover hippocampal physiology, in a remarkably packed account by Schwartzkroin and Mueller (primarily derived from studies on rodents), and hippocampal anatomy (mostly on the primate, by Rosene and Van Hoesen). The other major innovation in this volume is the chapter (Fallon and Loughlin) on monoamines, which certainly was a curious omission in the past. The other aspects covered are the role of glial cells (Somjen), various matters related to acetylcholine (3 chapters by Eckenstein and Baughman, Sillito and Murphy and Kristt), the colocalization of GABA with peptides (Jones, Hendry and DeFelipe), and the numbers of neurons and synapses in visual cortex (Peters).

Each chapter starts with a more or less comprehensive historical review. These are much more readable than many such accounts I have come across. However, they would have been better still if the flow of the text had not been interrupted, often in midsentence, by strings of references cited by author and date running up to 18 lines in length. This obviously is a problem faced in any review. Citations by number have their own problems, and I think it is entirely reasonable to decide against them. However, the worst excesses of the present system surely could have been avoided by the judicious use of footnotes. I certainly do not want to detract from the quality of the substance of the work. Much of the liveliness in the text itself stems, I suspect, from deliberate attempts by the authors to look forwards as well as backwards All the chapters include some attempt at a synthesis of the material reviewed, and some have bold and imaginative speculations on the functions of their particular systems in the cortex and thence in the animal as a whole.

There is much important material in this book. I feel it deserves a more detailed index, and some indication of the contents of the previous volumes. There is remarkably little cross-referencing, even within the present volume; this certainly makes life more difficult for the reader, and has allowed some inconsistencies to creep in (e.g., in the accounts of the cellular actions of the monoamines). The production of the book is to a high standard: good quality paper and decent print. The most successful figures were those specially (re)drawn; some others which were reproduced from earlier works could suffer from inconsistencies of style and labelling. However, all my reservations are minor quibbles; without a doubt this is a valuable addition to an excellent series.

JOHN G. R. JEFFERYS

Motor Areas of Cerebral Cortex. Edited by R. Porter. Ciba Foundation Symposium No. 132. 1987. Pp. 322. Chichester: John Wiley. Price £28.95.

Several times every year the Ciba Foundation provides funds for a 2-3 day gathering at its London headquarters of a small group of leading scientists and clinicians working in a common field. In exchange for the Foundation's generous hospitality, the participants not only write a short summary of their own contributions, but also allow publication of the comments they make during each discussion period. The meetings are not open to the public so that the only way to know what happened behind the closed doors of Portland Place is to read the books which emerge.

I found this book fascinating, not only for the quality of the individual chapters, some of which may be recommended as excellent short reviews in their own right, nor just for the illuminating periods of discussion which follow each chapter. Taken as a whole, the book provides a snapshot of current thought on the motor areas of the brain and it is this which provides the most important message of the book. The techniques may dazzle and the inventiveness thrill, but ultimately I confess to being a little disappointed at the present state of the motor soul. The matter is best illustrated by the fact that while reading the book, two colleagues independently volunteered the information that Professor Charles Phillips' chapter was the best in the book. Now, although Professor Phillips' chapter is a model of clarity and fair-mindedness, it is a historical review of the subject, which begins in the late nineteenth century and ends in the late 1960s. It is, quite purposely I must add, twenty years out of date! The reasons that this does not matter is that the anatomical and electroanatomical approach summarized by Professor Phillips is very much in the forefront of present research. The anatomy of the cortical areas, where they are, their interconnections and their output targets are what this book is all about. Physiological and psychological approaches to function take a second place.

The investigators of function lose because they offer us only one main insight over and above electroanatomical description: the distinction between mechanisms involved in internally and externally (i.e., visual, auditory or somatosensory) guided movements. Apart from this there is little information or speculation on what guiding principles the brain might use to organize movement. It is as though the ability to record single cell activity in awake moving animals which was pioneered by Ed Evarts in the 1960s has not provided insights in the motor system comparable to those obtained in the visual system. We can conceive how an image may be broken down into colours, contours and motion, but we have little idea of how the brain assembles a movement. You can imagine that, as a physiologist, I ended up disappointed with this final unwritten message.

Setting aside personal bias then, there is much 'that is very good about this book. The rapid publication is impressive, being only some six months after the meeting itself. I am not certain how this was achieved, but I suspect that it was because manuscripts were written before the meeting and handed in on arrival. This approach is to be commended for speed, but leads to intriguing discrepancies between the written account and the questions asked by participants. Where *are* some of the fascinating illustrations that Dr Thach and Dr Tanji showed? The discussions also provide details of the personal relations between some of the participants. It is comforting to know that even amongst the most august intellects there are still characters who, either through ignorance or prejudice, persistently aggravate their neighbours. This meeting dealt gently with two such outcasts by ignoring any comment passed by either of them. Human frailty also is evident. After a particularly hard grilling about the hypothesis put forward in his talk, one participant protested that he was only being provocative, as requested by the organizers. It must have been an enjoyable meeting.

JOHN ROTHWELL

Neuromodulation. The Biochemical Control of Neuronal Excitability. Edited by Leonard K. Kaczmarek and Irwin B. Levitan. 1987. Pp 286. Oxford University Press. Price £32.50.

Neuromodulation is a concept that has been in existence for many years, generally lacking a precise definition. This excellent book sets out to define the concept as 'the ability of neurones to alter their electrical properties in response to biochemical changes resulting from synaptic or hormonal stimulation' The 15 contributors have produced 13 well-presented, excellently illustrated chapters which begin with 2 chapters by the editors The first is on neuromodulation in which they define their concept and cite some examples of mechanisms which are treated in greater detail later in the book. The second chapter is an excellent résumé of ion currents and ion channels. The following chapters by Miller and by Huganir concentrate on theoretical and biochemical properties of channels and in particular the actions of kinases. The next 6 chapters of the book concentrate on particular ion channels and specific regulatory mechanisms for the control of neuronal firing. Many of the examples given are derived from studies on invertebrates such as *Aplysia* and *Helix* and overall this part of the book represents a comprehensive review of modulatory effects on ion channels in invertebrate neurons.

The final 3 chapters concentrate mainly on vertebrate studies with an excellent piece by Roger Tsien who discusses the modulation of calcium channels in heart cells and neurons.

The last 2 chapters on 'Neurotransmitter release and its modulation' and on 'Neuromodulation in the mammalian brain' were the most disappointing in the book. The penultimate chapter provides a very theoretical treatment of the calcium hypothesis of transmitter release with little new data after 1985, and the last chapter, the shortest in the book, gives only a very superficial outlook of synaptic modulation in the mammalian CNS. All the chapters are well produced, with a comprehensive bibliography at the end of each. One minor criticism is related to the quality of some of the illustrations, which have not all reproduced well from their original papers.

The editors, Leonard Kaczmarek from Yale and Irwin Levitan from Brandeis, are to be congratulated on producing such a timely and excellent volume. At £32.50 for its 290 pages it represents very good value. Unfortunately, as is the case with most specialist texts, the field, and this area of research in particular, is moving forward very quickly so that even now more information is available. Nevertheless it should be a must for the shelves of all neurophysiologists and is a splendid text to recommend for final year undergraduate courses in neuroscience.

S. D. LOGAN

ANNOUNCEMENTS

Fourteenth World Congress of Neurology

This will be held in New Delhi on October 22-27, 1989. Further information may be obtained from the Secretariat to the XIVth World Congress of Neurology, Department of Neurology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Joint Convention of the Fifth International Child Neurology Congress and the Third Asian and Oceanian Congress of Child Neurology

This will be held at the Nippon Convention Center on November 4-10, 1990. Further information may be obtained from the Secretariat to the Joint Convention of the 5th ICNC and 3rd AOCCN, c/o Simul International Inc., Kowa Building No. 9, 8-10 Akasaka 1-chome, Minato-ku, Tokyo 107, Japan (tel. (03) 586-8691).

Ninth International Congress of Pre and Perinatal Psychology and Medicine

This congress will be held at the Hilton Hotel, Jerusalem, Israel, on March 26-31, 1989 Further information may be obtained from the ISPP Congress Secretariat, c/o International Ltd., PO Box 29313, 65121 Tel-Aviv, Israel (tel. 654541).

Eighth Annual Meeting of the National Academy of Neuropsychologists

This will be held at the Sheraton World Resort, Orlando, Florida, on November 3-5, 1988. Further information may be obtained from Dr Antonio E Puente, Department of Psychology, University of North Carolina at Wilmington, Wilmington, NC 28403, USA (tel (919) 395-3812).

Journals of The British Psychological Society

British Journal of Psychology
Edited by D. E. Blackman, University College Cardiff
The Journal publishes reports of empirical studies likely to further our understanding of psychology; critical reviews of the literature; and theoretical contributions. Readership is world-wide and includes academic and research workers in experimental and cognitive psychology.
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British Journal of Medical Psychology
Edited by Sidney Crown, The London Hospital
The British Journal of Medical Psychology publishes original contributions to knowlege in the area of those aspects of psychology applicable to medicine and related clinical disciplines, including the psychotherapies and counselling Published articles include empirical studies, theoretical papers and clinical reprts which suggest increased understanding of the psychological aspects of medicine.

The readers are medical psychologists, mainly clinicians, concerned with applying psychology to medical problems, from the disciplines of psychology, medicine and professions allied to medicine.
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British Journal of Social Psychology
Edited by A. S. R. Manstead, University of Manchester
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British Journal of Developmental Psychology
Edited by Peter Bryant, Watts Professor of Psychology, University of Oxford
This new journal, launched in 1983, publishes authoritative scientific articles describing all aspects of development from infancy to old age, including educational implications and the problems of abnormal development
Volume 6 (1988) (4 parts) £45.00 (US$81.00)

British Journal of Mathematical and Statistical Psychology
Edited by A. D. Lovie, University of Liverpool
The Journal publishes articles relating to any areas of psychology which have a greater mathematical or statistical or other formal aspect to their argument than is usually acceptable to other journals Articles with a clear reference to substantive psychological issues are preferred, e.g. new models for psychological processes, new approaches to existing data, critiques of existing models and improved algorithms for estimating the parameters of a model.
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Journal of Occupational Psychology
Edited by David Guest, Department of Industrial Relations, London School of Economics
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British Journal of Clinical Psychology
Edited by Ray Hodgson, Department of Clinical Psychology, Whitchurch Hospital, Cardiff
The British Journal of Clinical Psychology publishes new findings, theoretical, methodological and review papers bearing on the whole field of clinical psychology and includes: descriptive and aetiological studies of psychopathology; applications of psychology to medicine and health care, studies of the assessment and treatment of psychological disorders; social and organizational aspects of psychological disorders and ill-health Contributions combine scientific excellence with relevance to the concerns of practising clinicians, bridging the gap between academic and professional interests.
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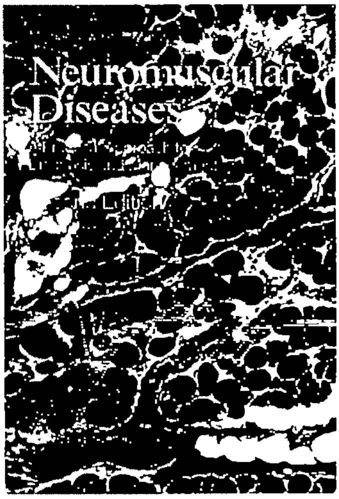

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